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ORIGINAL ARTICLES

NONTOXICITY AND ANTIPYRETIC EFFICIENCY OF TOLYSIN (ETHYL ESTER OF PARAMETHYLPHENYLCINCHONINIC ACID)*

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AMONG the drugs most useful as antipyretics and antirheumatics, recent investigations have given prominence to the phenylcinchoninic acid group. Of these the acid itself and the Ethyl Ester of its paramethyl derivative are marketed under the Council-accepted name cinchophen (also under the proprietary name atophan) and Tolysin respectively. Emphasis was originally placed upon their capacity to increase the elimination of uric acid.

Hanzlik, Scott and others[1], as well as Chace, Myers and Killian[2], have however demonstrated their efficiency in rheumatic fever and allied conditions. Here the two drugs exhibit reduction of temperature and relief of pain, apparently in the same manner and degree as salicylates. It has, therefore, become of importance to determine more definitely than is at present known, the comparative toxic as well as antipyretic values of the phenylcinchoninic acid derivatives in relation to each other as well as to salicylates. In the accumulation of such data the relatively low toxicity of the ester became so apparent that attention was centered chiefly upon this drug.

The preparation of ethyl ester of paramethylphenylcinchoninic acid used in this work is known commercially as Tolysin. The drugs compared with it were cinchophen and aspirin.

The results on animals will be reported under the respective headings "Toxicity tests," "Antipyretic effects," and "Experiments accounting for the nontoxicity of Tolysin."

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Toxicity Tests of Tolysin, Cinchophen, and Aspirin

MINIMUM LETHAL DOSE IN RATS

In these experiments acacia suspensions were used chiefly, each 100 c.c. containing 20 G. of drug. The injections were subcutaneous.

Tolysin.—The following doses exhibited no effect: 1, 2, 4, 10, and 10 G. per kilo (except that the 4 G. dose produced slight temporary lethargy). Twenty G. per kilo produced no effect on the day of injection, but the animal showed depression on the following day and died within 48 hours.

Cinchophen.—While 1 G. per kilo exhibited no effect, all larger doses were fatal. The following doses per kilo were given, and after each is mentioned the time within which death occurred: 2 G., 40 hours; 3 G., 36 hours; 4 G., 21 hours; 4 G., 40 hours; 8 G., 12 hours.

Aspirin.—One gram per kilo produced no result in one rat, but in another death occurred within 48 hours. The following fatal doses per kilo were also given: 2 G., 36 hours; 4 G., 20 hours; 8 G., 12 hours.

The rats were all old stock in excellent condition before injection. Absorption was rapid, if one may judge by the rate of reduction of the swelling.

Tolysin per os.—In four further experiments tolysin in doses per kilo of 2, 4, 10, and 10 G., respectively, yielded no effect, except for slight depression in one of the two receiving the largest dose.

From the above it appears that aspirin and cinchophen are at least ten times as toxic for rats as tolysin.

MINIMUM LETHAL DOSE FOR DOGS

The discrepancy between the toxicity of the ester tolysin and the acid cinchophen, appeared so significant that more extensive studies upon both drugs were undertaken. So far as tolysin is concerned, apparently no literature exists on this subject. As regards cinchophen, Starkenstein[3] placed the m. l. d. for dogs at 1.5 G. per kilo; while in the case of aspirin, Block[4] found that two administrations of 0.7 G. per kilo each were required to kill. Chidiehimo[5] places the m. l. d. at 0.5 G. per kilo. Neither of these investigators, however, was able to exclude vomiting.

In our first series of experiments hourly doses of the drugs were administered *per os,* in the form of aqueous acacia suspensions. The results may be summarized as follows (dosage per kilo being given):

Tolysin.—

1 x 5	G. =	5 G.	No effect.
12*x 1.67	G. =	20 G.	No effect.
7 x 4.29	G. =	30 G.	No effect.
6 x 5	G. =	30 G.	Slight incoördination and depression. Full recovery in twelve hours.
7 x 7.14	G. =	50 G.	No effect.

In the last cited experiment 275 grams (or over ½ lb.) were given to a 5½ kilo dog without effect.

*On two successive days, 6 doses each.

Cinchophen.—

3 x 0.33 G. = 1 G.	Depression; refused food. Recovered.
5 x 0.2 G. = 1 G.	Depression. Recovered in 12 hours.
5 x 0.25 G. = 1.25 G.	Marked depression; died within 12 hours.
5 x 0.33 G. = 1.5 G.	Marked depression. Died within 12 hours.

Aspirin.—In this series of aspirin experiments, vomiting occurred in every case, although in half of them the drug was administered in formaldehyde-hardened gelatin capsules, as indicated by the letters F.-G. Where vomiting occurred in these cases, the capsules were usually returned intact, so that the approximate proportion retained could be estimated.

The following doses were recovered from:

3 x 0.17 G. = 0.5 G.	(F.-G.);	(part retained).
2 x 2.02 G. = 0.5 G.	(F.-G.);	(⅛ retained).
1 x 0.5 G. = 0.5 G.	(F.-G.);	(⅛ retained).
1 x 0.5 G. = 0.5 G.	(F.-G.);	(⅕ retained).
5 x 0.15 G. = 0.75 G.;		(⅕ retained).
1 x 1 G. = 1 G.;		(part retained).
6 x 0.17 G. = 1 G.;		Marked depression; died within 12 hours.

The following fatalities occurred:

5 x 0.2 G. = 1 G. (Total 2 G.)	(part retained).	(Same dog as last—second day). Died.
6 x 0.17 G. = 1 G. (F.-G.)	Depressed. Died within 40 hours.	(½ retained).
7 x 0.21 G. = 1.5 G.	(part retained). Marked depression.	Died within 12 hours.

Owing to the amount of vomiting in each case, it was impossible to determine how much of the drug was retained. So that, from the above results one can only judge that 1 G., if absorbed, would be fatal. The experiment in which one gram was given and just half of the capsules retained, resulted in a slow (40 hours) fatality; whence it seems probable that one-half gram per kilo is the minimum lethal dose.

Chronic poisoning.—In the next series of experiments it was planned to compare the toxicity of the three drugs by giving smaller doses four times a day over a number of days. For this purpose the following doses per kilo were selected:

Tolysin	1 G. — 4 t. i. d.
Cinchophen	0.2 G. — 4 t. i. d.
Aspirin	0.1 G. — 4 t. i. d.

Vomiting was absent in this series. The tolysin was continued over six days, giving a total dose of 24 grams per kilo, with absolutely no effect upon the general condition of the animal. The cincophen dog showed some weakness at the end of the first day, gradually increasing until on the morning of the third day it was unable to stand. It was found dead on the morning of the fourth day—2 grams per kilo having been given in all. At autopsy a unilateral bronchopneumonia was found, which may have been a contributing factor.

The aspirin dog retained all of the drug; which rarely occurs with the dose given. The four doses sufficed to produce toward the end of each day definite signs of intoxication, including complete inability to stand and shallow rapid respirations. Each following morning he had apparently quite recovered.

The same phenomenon was repeated five days in succession.* It will be noted that the quantity of aspirin given was ten times less than that of tolysin, which under the same conditions, proved inert.

A 24-hour series.—In view of the above results it was thought that had the aspirin administration been continued at similar intervals overnight it would have been possible to produce in the blood a sufficient concentration of salicyl (or salicyl plus aspirin) to give a fatal result. A series of administrations was, therefore, conducted over a period of twenty-four hours, with eight three-hour intervals—making nine doses in all. In each case the individual doses were the same as in the series reported immediately before.

Tolysin was given in this way to one dog, and no effect followed a total of 9 grams per kilo in the 24 hours. Two dogs received cinchophen, both becoming extremely weak toward the end of the experiment. One died within 48 hours, but the other recovered after depression lasting four days. Hence, the minimum lethal dose of cinchophen, distributed over 24 hours, lies close to 1.8 G. per kilo.

Two dogs received in the same way a total of 0.9 G. per kilo of aspirin; but some vomiting followed each dose and both dogs recovered.

This series emphasizes further the difficulty of determining the m. l. d. of aspirin for dogs, on account of poor retention. Tolysin, however, was tolerated perfectly under the conditions, in ten times the dosage of aspirin which was rejected.

SUMMARY OF TOXICITY EXPERIMENTS IN DOGS

Tolysin produces neither vomiting nor other effects upon the general condition, even with doses up to 50 grams per kilo. It appears impossible to kill a dog with tolysin administered *per os.*

Cinchophen produces vomiting occasionally with oral doses upwards of 0.5 gm. per kilo. Four doses of 0.2 G. per kilo in a single day produced marked depression. The minimum lethal dose administered within eight hours is approximately 1.25 grams per kilo.

Aspirin in oral doses of 0.1 gram per kilo usually, and 0.2 gram per kilo constantly, causes vomiting. The former dose, if retained, produces marked depression after it has been repeated three or four times. A surely lethal dose is 1 gram per kilo, and the m. l. d. is probably 0.5 gram per kilo.

ABSENCE OF ALBUMINURIA IN TOLYSIN-TREATED DOGS

In three different dogs, given repeated therapeutic doses of tolysin, the urine was tested for albumin. The animals received: (1) 0.5 gram per kilo daily for four days, omitted for five days, then repeated daily for another four days; (2) 1 gram per kilo four times daily for six days; (3) 1 gram per kilo every three hours for nine doses.

In each case, the urine collected over the entire period of administration was negative for albumin.

*In an experiment of H. P. Foran the same dose given three times a day produced a temporary depression with convulsions at the end of the third day.

ANTIPYRETIC EFFECTS OF TOLYSIN, CINCHOPHEN, AND ASPIRIN

Since analgesic and antirheumatic effects cannot be satisfactorily determined in animals, the index of therapeutic efficiency of salicylates and drugs of the tolysin type must be their antipyretic action. In dogs this can be well shown by producing, with concentrated *B. coli* vaccine, a continuous fever, which usually persists for 48 hours.

Normal Dogs.—Like other antipyretic drugs, tolysin does not reduce the temperature of normal dogs. Although 50 grams per kilo fail to affect visibly the general behavior of a normal dog, slowing of the pulse may be demonstrated after one or two grams per kilo. This was the only significant effect seen in two dogs.

The first weighed 5.5 kilos, and exhibited, fasting, a normal pulse rate of 96. 5.5 grams tolysin *per os* was followed within one hour by a fall in pulse rate to 72. In a second dog, weighing 10 kilos, oral administration of 20 grams tolysin reduced the pulse rate within 1½ hours from 108 to 96 per minute.

As a result of the manipulation each dog exhibited a temporary increase in pulse and respiratory rates.

The first dog's temperature was unchanged, while that of the second showed a temporary increase of 0.7° C.

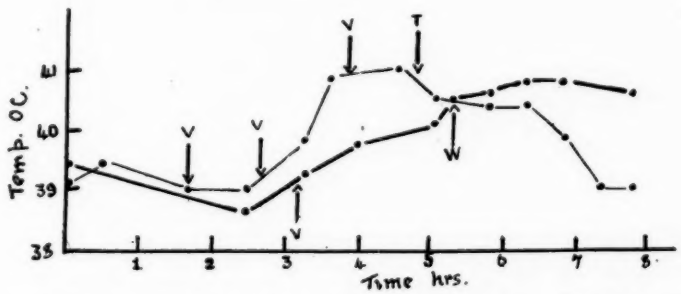

Fig. 1.—Antipyretic effects of tolysin in fever dog. *Light line:* Tolysin per os, 1.5 gm. per kilo, plus 250 c.c. water at *T*. *Heavy line*: Control Dog. 250 c.c. water only at *W*.
V.—0.1 c.c. per kilo typhoid-paratyphoid vaccine (intravenous).

Antipyretic Tests.—As no *B. coli* vaccine was at first available, we began with intravenous injections of a mixed typhoid-paratyphoid vaccine.* Injections of one or more tenths of a c.c. per kilo of this vaccine produced within 3 hours an increase of temperature amounting to from 1° to over 2° C. Unfortunately, even though the injection be repeated two or three times, the temperature usually falls without further interference almost as rapidly as it has risen; so that the determination of antipyretic efficiency becomes difficult.

To each of four dogs thus treated was administered *per os* 1 gram per kilo of tolysin, with the result that the temperature fell more rapidly than in any of the untreated animals. Two experiments with 1.5 grams per kilo yielded similar results; one of these is illustrated in Fig. 1, which shows also the curve of a control dog given 250 c.c. of water under the same conditions. The latter

*Lederle's "Typhoid combined Prophylactic No. 2", containing per c.c. *B. Typhosus* 1,000 million, *B. Para "A"* 500 million and *B. Para "B"* 500 million.

exhibited no reduction in temperature, at least until the tolysin had reduced to normal the temperature of the other dog.

For more exact determination of antipyretic efficiency the *B. coli* vaccine (one million million killed bacilli per c.c.) was found preferable. In two preliminary experiments this vaccine was injected at 9:30 A. M.; five hours later 1 gram of tolysin per kilo was given *per os.* In the first dog the temperature, which had increased by 1.2° C., was reduced to normal by tolysin within 2 hrs.,

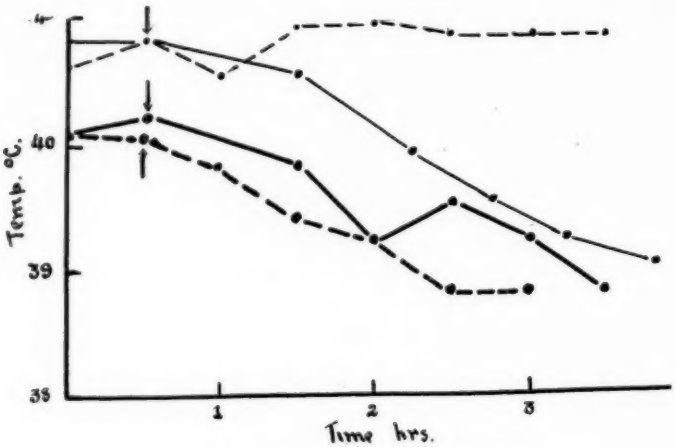

Fig. 2.—Antipyretic effects of tolysin in coli fever dogs; day after injection.
Light broken line: control (150 c.c. H_2O). *Light solid line*: tolysin 0.75 gm. per kg. at arrow. *Heavy solid line*: tolysin 1 gm. per kg. at arrow. *Heavy broken line*: composite curve from two experiments; tolysin 0.3 gm. per kg. at arrow.

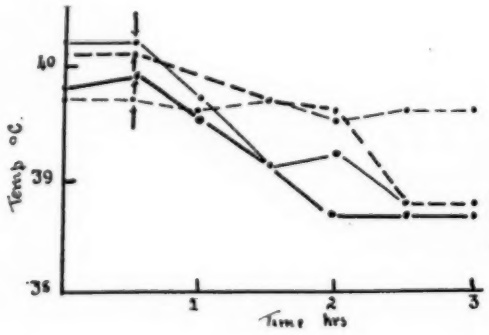

Fig. 3.—Antipyretic effects of tolysin, aspirin and cinchophen in coli fever dogs; day after injection.
Light broken line: control 150 c.c. H_2O. *Light solid line*: 0.15 gm. cinchophen per kg. at arrow. *Heavy solid line*: 0.1 gm. A. S. A. per kg. at arrow. *Heavy broken line*: 0.3 gm. tolysin per kg. at arrow.

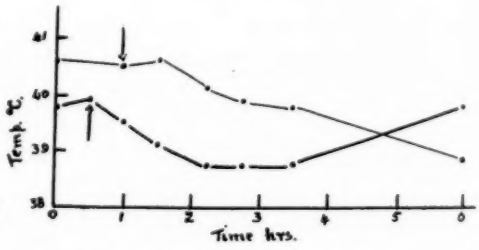

Fig. 4.—Persistence of tolysin effect.
Light line: 0.5 gm. tolysin per kg. at arrow. *Heavy line*: 0.1 gm. A. S. A. per kg. at arrow.

25 mins.; in the second animal recovery from a rise of 1.3° C. occurred within one-half hour of the injection. All of the subsequent experiments were performed upon the second fever day when the high temperature was found more stable.

To determine the minimal effective dose, gradually diminishing amounts were administered. We succeeded in reducing to normal the temperature of

two different *Coli* fever dogs by giving 0.3 gram per kilo. Smaller doses than this—e. g., 0.2 gram per kilo—were ineffective. For comparison, a number of fever dogs were treated with cinchophen and aspirin.

Antipyresis produced by doses of tolysin varying from 1 gm. down to 0.3 gm. per kilo is shown in Fig. 2. The maximum effect was obtained in from two to three hours after administration.

The respective effects of tolysin (0.3 gm. per kilo) cinchophen (0.15 gm. per kilo) and aspirin (0.1 gm. per kilo) may be compared in Fig. 3. It will be noted that the same degree of antipyresis was ultimately induced by all, but also that the two acids acted more promptly, although given in smaller doses.

Tolysin, the ester, is evidently more slowly absorbed. Compensating for this however is its more persistent effect, as seen in Fig. 4. Here is seen a prolonged antipyresis from 0.5 gm. per kilo tolysin, although 0.1 gm. per kilo aspirin acted more promptly.

While the minimal antipyretic dose of aspirin or cinchophen was found to be as low as 0.02 gm. per kilo of either, the effects produced thereby were submaximal and fleeting. The acute antipyretic experiment in the dog does not therefore afford the best criterion of comparative efficiency, owing to the differences in the rate of absorption. Where a more lasting effect is sought, as in acute rheumatic fever in man, the ratio of 15 gm. tolysin to 10 gm. aspirin or cinchophen appears to hold.

Even judging by the minimal antipyretic dose, in dogs tolysin presents a much more favorable $\frac{\text{"curative dose}}{\text{tolerated dose"}}$ ratio than do the acids in question: $\left(\frac{C}{T} = \frac{\text{minimal antipyretic dose}}{\text{minimal lethal dose}}\right)$

Tolysin	$\frac{C}{T}$	$=$	$\frac{0.3}{>50}$	$< \frac{1}{150}$
Aspirin	$\frac{C}{T}$	$=$	$\frac{0.02}{0.5}$	$\frac{1}{25}$
Cinchophen	$\frac{C}{T}$	$=$	$\frac{0.02}{1.25}$	$= \frac{1}{63}$

So that, even were 50 gm. per kilo fatal and the antipyretic effect of 0.3 gm. per kilo a fleeting one, tolysin would still have six times the "therapeutic range" of aspirin.

EXPERIMENTS ACCOUNTING FOR THE NONTOXICITY OF TOLYSIN

The finding of such a very large therapeutic range of dosage for tolysin led us to the conclusion that the rate of absorption must become considerably retarded as the dose is increased. This was put to the test by determining the amount of tolysin that could be recovered from the feces of dogs after various doses had been administered. This was done in three dogs given respectively: 0.5 gm. per kilo tolysin in one dose; 1.5 gm. per kilo in 7 hours; and 9 gm. per kilo in 24 hours. Additional tests were made in a case of acute

TABLE I

ABSORPTION OF TOLYSIN

EXPERIMENT	TOLYSIN		CONDITIONS	DETERMINATIONS	TOLYSIN POSSIBLE IN SAMPLE	PARAMETHYL CINCHOPHEN RECOVERED	TOLYSIN EQUIVALENT	ABSORBED (Calculated)	
	Gm.	Gm. per kilo		*	Gm.	Gm.	Gm.	Gm. p. kilo	Per Cent
Control A	1.5	-	mixed with 13.5 G. dog feces	2	0.50	0.40	0.44	-	-
Control B	12	-	mixed with 12 G. dog feces	2	5.00	4.63	5.09	-	-
Man (Ac. Rheum. Fever)	24	*0.3*	per os in *48 hours*	2	1.97	0.00	0.00	*0.3*	100
Dog No. 3A	2.75	*0.5*	per os in *one dose*	2	0.65	0.00	0.00	*0.5*	100
Dog No. 41A	6	*1.5*	per os in *7 hours*	2	1.72	1.04	1.15	*0.5*	33
Dog No. 44	45	*9*	per os in *24 hours*	3	5.69	3.60	3.97	*2.7*	30

*The results are based on averages from the given number of determinations.

Note:—A control "after-period" followed each absorption experiment except Dog No. 44, in which collection was continued over four days. No paramethylcinchophen was recovered in the after-periods.

rheumatic fever in man, where 0.3 gm. per kilo tolysin was given in 48 hours. Two control experiments were performed *in vitro*. In the first 1.5 grams tolysin was thoroughly mixed with 13.5 gm. dried dog feces; in the second 12 gm. tolysin was mixed with an equal amount of dried dog feces.

Method.—The following procedure (for the details of which we are indebted to a personal communication from Dr. M. S. Fine) was employed:

A 5 or 10 gram sample of dried feces was extracted with ether in a Soxhlet apparatus. This extract was saponified, using for each gram of extract 1½ grams KOH and 25 c.c. alcohol. Digestion was continued for about two hours on a water-bath, under reflux. After filtration, the filter paper was thoroughly washed with boiling alcohol and the alcoholic solution of soap was concentrated to a syrup; a minimum of distilled water was added and the solution boiled until all alcohol was removed.

The soap was then decomposed with dilute HCl, using methyl orange as an indicator. The fatty acid and paracinchophen were filtered off and dried together with the filter paper in a drying oven. The fatty acids melted and were absorbed by the paper, it being then possible to transfer quantitatively the paracinchophen to a weighed Gooch crucible. The paracinchophen was further purified by washing with benzol until free from fatty acids.

Control Experiments.—As will be seen from Table I, the tolysin was recovered quantitatively from the dried feces in a sample where 5 grams were present. In the smaller sample of the 1:9 mixture only 88 per cent of a possible half-gram were recovered. The error, therefore, may be as great as 12 per cent when such small quantities are dealt with. This will be found of no significance in drawing the chief conclusions from the following results:

Absorption Experiments.—The data from the case of acute rheumatic fever and the three dog experiments confirm the conclusions drawn from the pharmacologic evidence; for doses giving the maximum therapeutic effect, e. g., 0.3 and 0.5 gm. per kilo, respectively in man and dog, were apparently completely absorbed, no portion being recoverable in the feces.

But when the smallest dog dose was tripled there was no increase in absorption, the absolute amount being only 0.5 out of a possible 1.5 gm. per kilo.

Even where 9 gm. per kilo were given only 2.7 gm. per kilo were absorbed, and this amount would undoubtedly have been much less if the administration had not been extended evenly in 9 doses over 24 hours.

It is only necessary to add that where very large doses are given it is possible to observe clumps of tolysin powder in the feces of dogs.

We find, therefore, in tolysin a drug exhibiting the coincidence that the maximum limit of absorption is practically identical with the full therapeutic dose—apparently a new and fortunate peculiarity in pharmacologic behavior.

CONCLUSIONS

1. Ethyl ester of paramethylphenylcinchoninic acid (tolysin), administered *per os*, even up to doses of 50 gm. per kilo, or 5 per cent of the animal's own weight, produces no effect upon the general condition of dogs. Aspirin, however, may prove fatal in the oral dose of 0.5 gm. per kilo; 0.4 gm. per kilo,

given over a period of 9 hours, produces marked depression. The m. l. d. of cinchophen *per os* in dogs is about 1.25 gm. per kilo.

2. The respective minimal antipyretic dose over minimal lethal dose ratios for dogs are approximately:

$$\text{Tolysin} < \frac{1}{150}; \qquad \text{Aspirin} \ \frac{1}{25}; \qquad \text{Cinchophen} \ \frac{1}{63}.$$

Tolysin therefore seems to be the least toxic of all substances of demonstrated antirheumatic efficiency.

3. Tolysin exhibits a peculiarity in pharmacologic behavior in that the maximum limit of absorption from the intestine coincides essentially with full therapeutic doses. As cumulation is evidently absent, indefinitely large amounts are nontoxic, at least for dogs.

The authors take pleasure in acknowledging the assistance of Mr. Edward Tolstoi of the former Department of Pharmacology of Yale University, who performed a number of the preliminary experiments.

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THE EXCRETION OF SUGAR IN THE URINE IN HEALTH AND DISEASE*

By Ludwig Kast, Hilda M. Croll and Victor C. Myers, New York City

THE collection of accurate information concerning normal urinary sugar excretion was first made possible by Benedict and Osterberg,[1] who described in 1918 a method of estimating the sugar of normal urine. With this method they[2] found in two normal men an average daily excretion of 0.8 and 0.5 gram reducing substance (sugar) on a low carbohydrate diet, 1.0 and 0.65 gram on a medium carbohydrate diet, and 1.5 and 1.1 grams on a carbohydrate-rich diet. They also demonstrated that in normal individuals there is an increased sugar output in hourly urine specimens taken after meals. Kast, Wardell and Myers,[3] using the same method, demonstrated this "glycuresis" after meals in normal individuals and in diabetic patients. They showed that while the urine of a diabetic patient may be "sugar-free" in the morning, or several hours after a meal, large amounts of sugar may be excreted in the urine voided soon after the eating of food.

For this reason it has been emphasized by the latter workers that the use of a qualitative copper test on a morning urine specimen might be quite misleading in studying the carbohydrate tolerance of a patient. On the other hand, the determination of the amount of reducing sugar excreted in twenty-four hours in a series of cases would appear to be of value both in providing more information on normal sugar excretion and in showing the possible influence of disease on such excretion. Data are given in the present paper covering these points.

The original method of Benedict and Osterberg[1] for the determination of sugar in normal urine has the disadvantage of requiring the preliminary removal of interfering nitrogenous substances from the urine by precipitation with mercuric nitrate and sodium bicarbonate. That this is a laborious and time-consuming procedure is of some consequence in a study requiring many determinations of sugar. More recently Benedict and Osterberg[4] have described a new method for the determination of reducing sugar in normal urine which does not require this preliminary treatment with mercuric nitrate. The method is based on the reducing action of the sugar on a carefully controlled amount of picric acid and sodium hydroxide, following the treatment of the urine with specially purified bone black; the interfering action of creatine and creatinine is prevented by the addition of acetone, which dissipates the color due to these two compounds, without greatly in-

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A preliminary report of these observations was made before the Society for Experimental Biology and Medicine, November 16, 1921, Kast, L., and Croll, H. M., Proceedings, 1921, xix, 84.

fluencing the color arising from the sugar. In order to obtain accurate results, it is essential that the amount of sugar in the urine used for the test should be between 0.5 and 1.5 mg., i.e., not far from the amount (1 mg.) present in the standard. Benedict and Osterberg's results found by this method agree very closely with those obtained by their former procedure.

We have compared these two methods and also those of Shaffer and Hartmann[5] and Myers[6] (our observations were completed before the appearance of the method of Folin and Berglund[7]), and have found close agreement between the picric acid methods of Benedict and Osterberg, with higher

TABLE I

COMPARATIVE RESULTS FOR SUGAR IN NORMAL URINE FOUND BY VARIOUS METHODS

CASE	DATE	ACETONE-PICRIC ACID METHOD	MERCURIC NITRATE-PICRIC ACID METHOD	SHAFFER CUPROUS TITRATION METHOD	MYERS PICRIC ACID METHOD
	1920	per cent	per cent	per cent	per cent
S.	11/23-4	0.041			0.082
B.	11/22-3	0.075			0.106
L. A. K.	11/28-9	0.040			0.066
L. A. K.	11/29-30	0.021			0.038
L.	11/28-9	0.022			0.037
P.	11/30-12/1	0.031			0.048
E. L.	11/30-12/1	0.044			0.093
	1921				
C. G.	1/14-15	0.044			0.063
C. G.	1/16-17	0.040			0.052
V. D.	1/18-9	0.114			0.132
V. D.	1/19-20	0.081			0.093
T. A.	5/4-5	0.131	0.123	0.072	
T. A.	5/6-7	0.124	0.120	0.108	
T. A.	5/7-8	0.081	0.089	0.050	
L. N.	5/3-4	0.417	0.485	0.460	
F. H.	5/25-6	0.057	0.048	0.027	
F. H.	5/27	0.017	0.020	0.008	
L. A. K.	5/26-7	0.033	0.029	0.011	
N. B. M.	6/2-3	0.055	0.044	0.019	
C. H.	3/15	0.244	0.290		
C. H.	3/15	0.086	0.088		
J. P.	3/14-5	0.176	0.173		
C. H.	3/20-1	0.037	0.046		
M. K.	3/20-1	0.041	0.042		
M. F. M.	3/20-1	0.097	0.104		

results by Myers' and lower results by Shaffer's method. (Table I.) The last named determination is based on the reduction of copper sulphate by the sugar (after removal of interfering substances with mercuric nitrate) and the iodometric titration of the cuprous oxide. Shaffer and Hartmann have shown that this gives lower results than the mercuric nitrate picric acid method of Benedict and Osterberg. Myers' method depends on the precipitation of creatinine from the urine by saturation with picric acid, and the reduction of an alkaline picrate solution by the sugar in the urine. It is probable that in some cases the removal of creatinine is not so complete as in others, and this would account for the irregularity of the higher figures obtained for sugar by this procedure.

Comparison was made also between the results obtained by the acetone-picric acid method and the Benedict[8] qualitative copper test for sugar. The qualitative copper test is sensitive to slightly less than 0.1 per cent reducing sugar in urine. It is seen in Table II that if the specific gravity of a normal urine is high, i.e., if the urine is concentrated, a higher percentage of sugar is necessary to give even a slight reduction of the copper reagent, than if the urine is more dilute. This is probably due to the interfering action of certain urinary constituents present in larger amount in the concentrated urines.

TABLE II

COMPARISON OF THE ACETONE-PICRIC ACID METHOD WITH THE BENEDICT QUALITATIVE COPPER TEST

CASE	DATE	URINE				
		VOLUME	SP. GR.	REDUCING SUGAR		
				ACETONE-PICRIC ACID METHOD		BENEDICT QUALITATIVE TEST
	(1920)	c.c.		per cent	grams	
R. B.	12/17-18	1560	1013	0.030	0.47	negative
L. M.	10/27-8	1190	1015	0.048	0.57	negative
C.	11/4-5	2000	1012	0.047	0.97	negative
W.	12/3-4	1260	1019	0.068	0.86	negative
T. B.	11/21-2	720	1025	0.090	0.65	negative
	(1921)					
L. H.	3/30-1	1570	1021	0.099	1.50	very sl. +
L. H.	3/31-4/1	1350	1023	0.098	1.32	very sl. +
L. H.	4/1-2	2180	1017	0.121	2.64	+
A. B.	3/23-4	1000	1020	0.107	1.07	very sl. +
McL.	1/30-1	780	1033	0.108	0.85	very sl. +
McL.	1/28-9	690	1034	0.136	0.94	very sl. +
B. J.	6/18-9	300	1026	0.140	0.42	sl. +
D.	1/25-6	600	1024	0.162	0.97	sl. +
L. E.	2/17-8	730	1028	0.122	0.89	sl. +
M. D.	4/15-6	600	1025	0.117	0.70	sl. +
T. B.	4/6-7	420	1029	0.198	0.83	+
M. C.	6/16-7	690	1016	0.290	2.00	+
M. C.	6/17-8	1110	1020	0.260	2.90	+
M. C.	6/19-20	1420	1013	0.300	4.30	+
C. O.	5/4-5	250	1031	0.265	0.66	+
C. O.	5/5-6	250	1039	0.303	0.76	++
L. N.	5/2-3	1188	1016	0.361	4.16	++
L. N.	5/3-4	1015	1019	0.417	4.20	++
L. N.	5/8-9	960	1019	0.316	3.04	++

That the normal sugar output is remarkably constant on an unvarying diet is shown in Table III, in which our daily findings in a few cases are recorded. It is seen also that a low carbohydrate diet results in a lower daily sugar excretion than a medium or high carbohydrate diet. These facts are in harmony with the findings of Benedict[2] and Folin and Berglund.[9]

The effect of the ingestion of food on the hourly sugar output is shown in one case in Table IV. While the hourly sugar excretion on the morning of December 15, 1920, was 37 gm., after a breakfast containing about 60 grams of carbohydrate, the output rose gradually to 59 mg. per hour; it fell to 34 mg. before lunch and rose after lunch to 76 mg. hourly. On March 2, 1921, during the hour before dinner, 26 mg. of sugar were excreted; after

TABLE III
THE EFFECT OF DIET ON THE DAILY SUGAR EXCRETION

DATE	CASE	SEX	DIAGNOSIS	URINE				DIET—HIGH, MEDIUM, OR LOW CARBOHYDRATE
				VOLUME	CREATININE	REDUCING SUGAR		
(1920)				c.c.	grams	per cent	grams	
11/15-6	T.	♀	Undernutrition, headaches, neurasthenia.	1536	0.933	0.039	0.60	Medium
11/16-7	"			1440	0.997	0.042	0.61	"
11/17-8	"			1600	0.875	0.034	0.60	"
(1921)								
3/30-1	M. B.	♀	Dyspituitarism, dysthyroidism.	1560	1.310	0.047	0.73	Medium
3/31-4/1	"			1580	1.320	0.048	0.76	"
3/23-4	M. K.	♀	Psychasthenia, hypotonia.	1710	1.120	0.047	0.80	Medium
3/24-5	"			1380	1.140	0.059	0.82	"
4/8-9	S. B.	♂	Gastric neurosis.	1320	1.810	0.083	1.10	Medium
4/9-10	"			960	1.890	0.125	1.20	"
6/17-18	J. S.	♂	Essential hypertension.	1990	1.360	0.062	1.23	Medium
6/19-20	"			1780	1.350	0.071	1.26	"
2/28-3/1	H. C.	♀	Normal.	1089	1.101	0.059	0.65	Sl. low
10/20-1	"			1520	1.095	0.039	0.59	Sl. low
10/19-20	"			1330	1.095	0.065	0.87	Medium
(1922)								
4/4-5	"			1925	1.115	0.040	0.77	Medium
4/5-6	"			1880	1.250	0.041	0.77	Medium
(1920)								
12/14-15	"			1747	.....	0.065	1.14	High
(1922)								
3/26-27	H. N.-l.	♂	Chronic myocarditis.	550	0.599	0.042	0.23	Very low
4/13-14	"			1020	0.630	0.060	0.61	Medium
5/12-13	K.-l.	♂	Chronic interstitial nephritis.	1820	0.650	0.100	1.80	High
6/8-9	"			1520	0.610	0.060	0.91	Low

dinner, and later after additional food was eaten, the hourly output rose to 84 mg.

Our plan was to determine the amount of reducing substance (sugar) in twenty-four hour urine specimens of normal individuals and hospital patients, using the technic of the new acetone-picric acid method of Benedict and Osterberg. In all cases the sugar was determined in several twenty-four hour specimens, and the accuracy of the urine collections was checked by determinations of the daily creatinine output. Note was made of the diets of the individuals and these were classified according to carbohydrate content. The daily output of sugar was comparatively constant when the diet contained about the same amount of carbohydrate each day; accordingly, in order to save space, the daily urine volumes and amounts of sugar excreted by each individual were averaged for Table V; the average figures there shown are much the same as the figures obtained for each of the individual days.

It was found that in twelve normal adults, from 0.62 to 1.23 grams of reducing sugar on the average were excreted in twenty-four hours. The average figure for the twelve cases was 0.96 gram. Neuwirth,[10] using the same method[4] of sugar determination in studying twenty-six normal individuals, has demonstrated a daily excretion of 0.941 gram reducing sugar on the average, with a range of 0.614 to 1.383 grams. Folin and Berglund,[8] using their new colorimetric method[7] for sugar in normal urine, determined the twenty-four hour amounts of sugar in the urine of a normal man. When he was on a diet of meat, eggs and 50 or 200 grams of pure glucose daily for eight days, the daily amount of reducing sugar was between 0.543 and 0.980 gram; when he was on a mixed diet, including starches, for six days, the twenty-four hour excretion of reducing sugar was from 0.885 to 1.650 grams. The average daily figure for the six days on a mixed diet was 1.203 grams reducing sugar. Folin and Berglund showed also that there is an increase in the reducing sugar in normal urine after hydrolysis, confirming the observation previously made by Benedict.[11]

We found an average daily excretion of 0.42 to 1.07 grams of reducing sugar in twelve patients diagnosed as neurasthenics. In twenty-four patients suffering from conditions associated with constipation, the average range was from 0.41 to 1.80 grams reducing sugar daily, with the exception of one man (M. H-e. Table V) who excreted 3.85 grams reducing sugar in one day. No reason was found for this relatively high sugar excretion, either in the diet or from the history. In six cases of hyperthyroidism an average of 0.46 to 1.61 grams reducing sugar were excreted daily. Four patients with dyspituitarism excreted daily from 0.69 to 1.18 grams reducing sugar on the average, while a patient suffering from hypothyroidism excreted 0.40 gram sugar, after a low carbohydrate diet. A woman having myxedema excreted on the average 0.62 gram reducing sugar daily. It has been shown by various investigators[12] that a high fasting blood sugar and low carbohydrate "tolerance," or high and delayed blood sugar curve after glucose ingestion, may exist in hyperthyroidism, and a low fasting blood sugar and high "tol-

TABLE IV

THE EFFECT OF FOOD INGESTION ON THE HOURLY EXCRETION OF SUGAR
CASE C. (NORMAL)

HOUR	FOOD	URINE				SUGAR CALCULATED HOURLY
		VOLUME	SP. GR.	REDUCING SUGAR		
		C.C.		PER CENT	MGS.	MGS.
(12/14/20)	8 A. M.					
A. M.	Breakfast					
9-11	(45 g. CHO)	100	1022	0.058	58	29
11-12		50	1021	0.055	28	
P. M.	1 P. M.					
12-1	Lunch	34	1024	0.064	22	
1-2	(50 g. CHO)	34	1024	0.073	25	
2-3		33	1027	0.099	33	
3-4		39	1028	0.091	35	
4-5		42	1029	0.083	35	
5-6	6 P. M.	24	1032	0.094	23	
	Dinner					
6-7	(120 g. CHO)	40	1030	0.101	41	
7-8		47	1031	0.103	49	
8-9.30	9 P. M.	106	1030	0.086	91	61
	Candy					
9.30-10.30	(60 g. CHO)	69	1028	0.105	72	
10.30-8 A. M.	8 A. M.	615	1015	0.057	352	37
	Breakfast					
(12/15/20)	(60 g. CHO)					
8-9		50	1018	0.083	42	
9-10		101	1017	0.058	59	
10-11		128	1016	0.043	56	
11-12	12.15 P. M.	104	1015	0.033	34	
P. M.	(50 g. CHO)					
12-1	1 P. M.	72	1019	0.058	42	
	Lunch					
1-4	(40 g. CHO)	326	1015	0.070	229	76
4-5		65	1018	0.086	56	
Total	About					
32	425 g. CHO	2079		0.065	1382	
2/28/21						
A. M.	Breakfast					
	(40 g. CHO)					
9-1	1 P. M.	335	1021	0.043	139	35
P. M.	Lunch					
1-2	(65 g. CHO)	70	1018	0.038	27	
2-4		83	1021	0.061	51	26
4-5		26	1029	0.110	29	
5-6		33	1022	0.083	27	
	6 P. M.					
6-7	400 c.c. H_2O	60	1023	0.082	49	
7-8		49	1022	0.077	38	
8-9		77	1018	0.054	42	
9-10	9.30 P. M	57	1017	0.045	26	
	Lunch					
10-10.30	(20 g. CHO)	14	q. n. s.	0.069	9.6	19
10.30-8		270	1017	0.073	196	21
	8 A. M.					
3/1/21	Breakfast					
A. M.	(40 g. CHO)					
8-9		15	q. n. s.	0.103	16	
9-10		15	q. n. s.	0.122	18	
10-11		50	1021	0.082	41	
11-12		37	1022	0.078	29	
Total	About					
27	165 g. CHO	1191		0.062	737.6	

TABLE IV—CONT'D

THE EFFECT OF FOOD INGESTION ON THE HOURLY EXCRETION OF SUGAR
CASE C. (NORMAL)

HOUR	FOOD	URINE				SUGAR CALCULATED HOURLY
		VOLUME	SP. GR.	REDUCING SUGAR		
3/2/21	8 A. M.	C.C.		PER CENT	MGS.	MGS.
A. M.	Breakfast					
9-11	(55 g. CHO)	108	1024	0.082	88	44
11-12	12 M.	53	1023	0.065	34	
	Milk					
12-1	(10 g. CHO)	31	1027	0.072	22	
1-2	1 P. M.	45	1027	0.077	35	
2-3	Lunch	53	1027	0.078	41	
	(65 g. CHO)					
3-4		54	1025	0.064	35	
4-5	4 P. M.	185	1015	0.028	52	
5-6	400 c.c. H_2O	105	1015	0.025	26	
	6 P. M.					
6-7	Dinner	72	1016	0.036	26	
	(55 g. CHO)					
7-8	7 P. M.	56	1016	0.051	29	
8-9	Fruit	63	1015	0.085	54	
	(25 g. CHO)					
9-10	8 P. M.	175	1010	0.048	84	
	Fruit					
	(15 g. CHO)					
Total	About					
13	225 g. CHO	1000		0.053	526	

erance'' are often seen in hypothyroidism and dyspituitarism. It is interesting to note that no great departure from normal daily sugar excretion was seen in our cases of these diseases.

In our series of thirteen patients with vascular hypertension, there was a daily average sugar excretion of 0.44 to 1.12 grams. Five patients with cardiac disorders excreted from 0.51 to 0.71 gm. sugar daily. In ten cases of nephritis we found an average range from 0.61 to 1.89 grams. Although in cases of severe nephritis a mild hyperglycemia is generally present, no essential change from normal sugar excretion was found in these cases. It appears that there is no lowering in the excretion of sugar in nephritis.

Carbohydrate metabolism may be disturbed in some way in arthritis, according to Pemberton.[13] We studied sugar excretion in seven cases of arthritis and found that the range of daily sugar excretion was between 0.44 and 1.39 grams. In three cases of rheumatism, from 0.80 to 1.23 grams of sugar were excreted daily.

It has been shown by various investigators that there is a hyperglycemia in carcinoma. We found no variation from the normal sugar excretion in six cases of abdominal carcinoma, with an average range of 0.62 to 0.81 gm. sugar daily.

In a series of hospital cases representing a variety of pathologic conditions, in which were included disorders of the stomach, uterus and gall bladder, and such diseases as eczema and encephalitis lethargica, no departure from normal sugar excretion was noted. The case of A. N-l. (Table V), a

TABLE V

AVERAGE DAILY SUGAR EXCRETION IN NORMAL AND PATHOLOGICAL URINES

DATE, MONTH AND YEAR	CASE	SEX	AGE	WEIGHT	DIET,* LOW, MEDIUM OR HIGH CHO	URINE				DIAGNOSIS
						NUMBER OF DAILY DETNS AVERAGED	VOLUME	REDUCING SUGAR		
			YEARS	KILOS			C.C.	PER CENT	GRAMS	
2-21	H. C-l.	♀	25	52	Sl. low	2	1305	0.048	0.62	Normal
	H. C-l.				Medium	3	1711	0.047	0.80	
	H. C-l.				High	1	1747	0.065	1.14	
6-22	L. De K-f.	♀	24	53	Low	1	465	0.140	0.65	Normal (Father had diabetes)
5-22	G. P-n.	♂	24	55	Medium	3	1510	0.055	0.83	Normal
4-22	L. M-r.	♀	45	61	Medium	4	1169	0.074	0.87	Normal
6-22	M. W-b.	♀	24	70	Medium	1	785	0.113	0.89	Normal
12-21	V. M-s.	♂	38	77	High	3	838	0.118	0.99	Normal
11-21	C. R-h.	♀	17	48	Medium	2	915	0.113	1.03	Normal
4-22	F. T-n.	♀	35		Medium	1	1910	0.054	1.03	Normal
12-21	H. B-e	♀	25	52	Medium	1	1560	0.068	1.06	Normal
3-21	A. S-s.	♀	35	70	High	2	2600	0.044	1.14	Normal
12-21	D. K-l.	♂	40	70	Medium	1	1010	0.118	1.19	Normal
10-21	M. C-h.	♀	27	64	High	3	725	0.170	1.23	Normal
10-20	J. L. F-y.	♀	35		Medium	1	890	0.046	0.41	Anemia
1-21	Mc L-n.	♀	17	55	Medium	6	840	0.090	0.76	Undernutrition
2-21	W-y.	♀	25	63	Medium	1	1440	0.063	0.90	Postoperative convalescence
2-21	M-e.	♀	41	61	Medium	1	1020	0.041	0.42	Psychasthenia
3-21	B. A. S-s.	♀	60	39	Medium	3	710	0.062	0.44	Psychosis
3-21	C. H-y.	♀	57	50	Medium	3	1120	0.052	0.58	Psychasthenia, hypotonia
11-20	T-r.	♀	44	56	Medium	3	1525	0.039	0.60	Neurasthenia, undernutrition
12-20	A-h.	♀	38	61	Medium	4	1031	0.060	0.62	Neurasthenia, optic neuritis
3-21	M. K-p.	♀	57	73	Medium	3	1670	0.047	0.80	Psychasthenia, hypotonia
12-20	C-e.	♀	57	70	Medium	2	1230	0.066	0.81	Neurasthenia
3-21	B-d.	♀	41	61	Medium	2	1560	0.052	0.82	Neurasthenia
12-20	W-r.	♂	62	68	Medium	3	1250	0.066	0.83	Neurasthenia, undernutrition
2-21	E-n.	♀	53	48	Medium	3	1190	0.072	0.86	Psychosis, anemia
5-21	P. C. B-e.	♂	31	68	Medium	3	1480	0.063	0.94	Hypotonia, psychasthenia
3-21	A. J. B-n.	♀	23	68	Medium	1	1000	0.107	1.07	Neurasthenia
11-20	L-y.	♀	61	68	Medium	1	1840	0.022	0.41	Constipation, psychoneurosis
12-20	G-n.	♂	64	64	Medium	1	1620	0.028	0.46	Constipation, diverticuli coli

*Low carbohydrate diet contains approximately 100 gm. carbohydrate daily, medium CHO diet about 250 to 300 gm., and high CHO diet over 300 gm. For children the amounts are smaller.

TABLE V—CONT'D

AVERAGE DAILY SUGAR EXCRETION IN NORMAL AND PATHOLOGICAL URINES

DATE, MONTH AND YEAR	CASE	SEX	AGE	WEIGHT	DIET,* LOW, MEDIUM OR HIGH CHO	URINE				DIAGNOSIS
						NUMBER OF DAILY DETNS AVERAGED	VOLUME	REDUCING SUGAR		
			YEARS	KILOS			C.C.	PER CENT	GRAMS	
5-21	E. R-n.	♀	48	54	Medium	3	760	0.066	0.50	Constipation, splanchnoptosis, anemia
3-21	R. B-e.	♀	26	50	Medium	3	1360	0.037	0.50	Constipation, chronic tonsillitis
1-21	N-t.	♀	37	45	Medium	3	1120	0.055	0.62	Constipation, neurasthenia
11-20	B-r.	♂	44	63	Medium	1	900	0.075	0.67	Constipation, neurasthenia
3-21	M. F. M-e.	♀	14	50	Medium	3	942	0.071	0.67	Constipation, colitis, neurasthenia
4-21	M. F-d.	♀	34	64	Medium	2	1740	0.041	0.71	Urticaria
1-21	E. L-e.	♂	38	66	Medium	7	1724	0.041	0.71	Constipation, autointoxication, undernutrition
3-21	F. S-l.	♀	60	60	Medium	1	1650	0.047	0.77	Constipation, anemia
12-20	R. G-k.	♀	40	64	Medium	4	1764	0.045	0.80	Constipation, neurasthenia
3-21	W. G. L-d.	♀	60	64	Medium	1	945	0.087	0.83	Colitis, autointoxication, pyelitis
4-21	H. E. V-n.	♂	60	77	Medium	6	2070	0.041	0.85	Constipation
4-21	J. A-r.	♀	59	63	Medium	2	1140	0.077	0.88	Constipation
1-21	G-a.	♀	38	55	Medium	4	2260	0.040	0.91	Colitis, autointoxication, neurasthenia
2-21	P. H. S-h.	♀	46	64	Medium	1	1000	0.096	0.96	Enteritis, constipation, psychoneurosis
11-20	C-t.	♀	42	60	Medium	1	2000	0.048	0.97	Constipation, colitis
1-21	D-l.	♀	35	53	Medium	5	1620	0.060	0.98	Constipation, psychoses, undernutrition
11-20	W-g.	♀	43	57	Medium	2	1455	0.069	1.01	Constipation, hemorrhoids, neurasthenia
4-21	H. S. McK-e.	♂	30	64	Medium	3	860	0.118	1.01	Autointoxication, hypotonia, undernutrition
4-21	V. E. M-y.	♂	50	66	Medium	3	1336	0.093	1.24	Autointoxication, psychasthenia
1-22	J. G-s.	♂	45	73	High	5	1418	0.119	1.69	Constipation
3-21	L. F. H-z.	♂	33	63	High	3	1700	0.106	1.80	Autointoxication, migraine
12-20	M. H-e.	♂	46	66	Medium	1	2790	0.138	3.85	Constipation, migraine, autointoxication, neurasthenia
3-21	W. H-h.	♀	24	50	Medium	1	550	0.083	0.46	Hyperthyroidism
2-21	H. R-n.	♂	18	54	Medium	6	964	0.064	0.62	Hyperthyroidism
6-21	R. M-s.	♀	22	49	Medium	2	660	0.130	0.86	Hyperthyroidism
2-21	G.	♀	40	60	Medium	7	1377	0.064	0.88	Hyperthyroidism
5-21	T. A-n.	♀	27	47	Medium	9	842	0.116	0.98	Hyperthyroidism
1-22	I. G-g.	♂	36	50	High	7	1683	0.096	1.61	Exophthalmic goitre, hyperthyroidism

*Low carbohydrate diet contains approximately 100 gm. carbohydrate daily, medium CHO diet about 250 to 300 gm., and high CHO diet over 300 gm. For children the amounts are smaller.

TABLE V—CONT'D

AVERAGE DAILY SUGAR EXCRETION IN NORMAL AND PATHOLOGICAL URINES

DATE, MONTH AND YEAR	CASE	SEX	AGE	WEIGHT	DIET,* LOW, MEDIUM OR HIGH CHO	URINE				DIAGNOSIS
						NUMBER OF DAILY DETNS AVERAGED	VOLUME	REDUCING SUGAR		
			YEARS	KILOS			C.C.	PER CENT	GRAMS	
4-21	R. C-e.	♀	31	53	Medium	2	1080	0.077	0.83	Dysthyroidism, neurasthenia
3-21	M. B-s.	♀	19	78	Medium	2	1570	0.047	0.74	Dysthyroidism, dyspituitarism
11-20	B-t.	♀	63	70	Medium	1	1620	0.043	0.69	Dyspituitarism, undernutrition, migraine
11-20	Mc. R-s.	♀	46	91	Medium	2	1845	0.057	1.05	Dyspituitarism, hypotonia, colitis
4-21	W. E-n.	♀	43	89	Medium	1	1620	0.073	1.18	Dyspituitarism, obesity
6-21	B. J-n.	♀	58	86	Low	3	460	0.087	0.40	Hypothyroidism, obesity, hypertension
1-22	I. B-k.	♀	52	86	Medium	5	1060	0.058	0.62	Myxedema
11-20	P-y.	♀	54	71	Medium	1	1440	0.031	0.44	Vascular hypertension, neurasthenia
8-21	G. S-l.	♀	52	86	Low	2	345	0.133	0.46	Essential hypertension
2-21	E. H-r.	♀	73	57	Medium	7	1046	0.049	0.51	Essential hypertension
1-21	F-n.	♀	50	50	Medium	2	1770	0.033	0.58	Vascular hypertension, neurasthenia, headaches
4-21	M. D-g.	♀	56		Medium	16	815	0.07[illegible]	0.59	Cardiac hypertrophy, hypertension
12-20	S-t.	♀	60	64	Medium	4	990	0.060	0.59	Vascular hypertension, colitis, neurasthenia
4-21	M. D-s.	♀	23	53	Medium	13	650	0.091	0.59	Hypertension, hysteria
2-21	E. F-n.	♀	48	84	Medium	6	1870	0.038	0.71	Essential hypertension
11-20	B-r.	♀	40	63	Medium	1	1680	0.054	0.91	Vascular hypertension, colitis
4-21	B. Y-r.	♂	53	69	Medium	7	1494	0.062	0.93	Essential hypertension
2-21	L. E-r.	♂	36	68	Medium	8	765	0.128	0.98	Essential hypertension
4-21	B. de C-r.	♀	40	60	Medium	2	2100	0.045	0.99	Vascular hypertension, neurasthenia, constipation, migraine
6-21	J. S-r.	♂	41	70	Medium	3	1790	0.062	1.12	Essential hypertension
5-21	F. H-l.	♀	30	54	Medium	4	1037	0.080	0.83	Migraine, hypotonia
2-21	B. G-d.	♂	27	58	High	9	988	0.062	0.61	Chronic diffuse nephritis
4-21	E. G-e.	♂	23	66	Medium	1	1260	0.052	0.66	Nephritis, secondary contracted kidney
3-21	P. R-o.	♂	41	64	Medium	6	1630	0.042	0.69	Chronic interstitial nephritis
6-21	J. S-r.	♂	17	44	Medium	1	1535	0.046	0.71	Chronic diffuse nephritis, complications
12-20	M-y.	♀	51	59	Medium	5	1626	0.048	0.78	Chronic nephritis, hypertension, cystitis
1-22	J. B-e.	♂	50	52	Medium	9	1329	0.062	0.82	Arteriosclerotic kidneys, cardiac hypertrophy and dilatation

*Low carbohydrate diet contains approximately 100 gm. carbohydrate daily, medium CHO diet about 250 to 300 gm., and high CHO diet over 300 gm. For children the amounts are smaller.

TABLE V—CONT'D

TABLE V—CONT'D

AVERAGE DAILY SUGAR EXCRETION IN NORMAL AND PATHOLOGICAL URINES

DATE, MONTH AND YEAR	CASE	SEX	AGE	WEIGHT	DIET,* LOW, MEDIUM OR HIGH CHO	URINE				DIAGNOSIS
						NUMBER OF DAILY DETNS AVERAGED	VOLUME	REDUCING SUGAR		
			YEARS	KILOS			C.C.	PER CENT	GRAMS	
6-21	J. S-k.	♀	38	63	Medium	4	1119	0.080	0.89	Chronic pyelonephritis
1-22	I. L-o.	♂	55	59	High	4	1325	0.094	1.25	Arteriosclerotic kidneys, hypertension
2-22	J. M-i.	♂	55	68	High	14	2045	0.084	1.72	Arteriosclerotic kidneys, cardiac hypertrophy and dilatation
5-22	S. K-e.	♂	26	60	High	10	1499	0.126	1.89	Chronic interstitial nephritis
6-21	S. B-y.	♀	45	52	Medium	4	537	0.095	0.51	Chronic cardiovalvular disease
4-21	M. M-y.	♀	50	51	Medium	9	432	0.127	0.55	Arteriosclerosis, gastritis
11-21	H. N-l.	♂	70	64	Medium	7	925	0.063	0.58	Chronic myocarditis
5-21	C. O'N-l.	♀	52	83	Medium	7	516	0.126	0.65	Auricular fibrillation, edema
3-21	J. F-r.	♂	48	73	Medium	3	1190	0.060	0.71	Auricular fibrillation
6-21	M. H-r.	♂	39	80	Very low	1	455	0.097	0.44	Osteoarthritis
1-21	B-n.	♀	53	66	Medium	2	2250	0.023	0.51	Chronic arthritis, eczema, neurasthenia, constipation
6-21	N. B. M-y.	♀	51	73	Medium	3	960	0.062	0.60	Arthritis
3-21	E. R-	♀	38	—	Low	1	1080	0.062	0.67	Arthritis deformans
7-21	R. C-s.	♂	24	65	Medium	6	757	0.117	0.89	Osteoarthritis
5-21	C. T-r.	♂	49	103	Very low	2	2641	0.040	1.07	Acute arthritis, chronic myocarditis
4-21	H. K-n.	♂	30	62	Medium	12	1013	0.123	1.39	Arthritis deformans, mitral stenosis and insufficiency
3-21	C-l.	♀	39	—	Medium	3	1450	0.055	0.80	Rheumatism
11-20	B-s.	♀	69	—	Medium	1	1560	0.071	1.09	Rheumatism, constipation, vascular hypertension
7-21	H. S-n.	♂	14	37	High	8	1177	0.105	1.23	Rheumatic fever, tonsillitis
1-21	Y-e.	♂	60	45	Medium	3	1980	0.031	0.62	Abdominal carcinoma, anemia
11-20	U. S-d.	♂	77	62	Medium	1	1620	0.041	0.67	Carcinoma of stomach, abdominal metastases
2-21	W. S. M-r.	♀	54	50	Medium	1	470	0.150	0.71	Carcinoma of sigmoid
2-21	I. S-r.	♀	52	76	Medium	1	1430	0.049	0.71	Intraperitoneal carcinoma
12-20	E. W-e.	♀	50	54	Medium	1	1820	0.040	0.73	Carcinoma recti
7-21	A. R-e.	♂	51	57	Medium	3	583	0.139	0.81	Carcinoma of liver and abdomen
3-21	L. A. K-n.	♀	31	61	Medium	10	1153	0.040	0.46	Achylia gastrica, neurasthenia

*Low carbohydrate diet contains approximately 100 gm. carbohydrate daily, medium CHO diet about 250 to 300 gm., and high CHO diet over 300 gm. For children the amounts are smaller.

TABLE V—CONT'D
AVERAGE DAILY SUGAR EXCRETION IN NORMAL AND PATHOLOGICAL URINES

DATE, MONTH AND YEAR	CASE	SEX	AGE	WEIGHT	DIET,* LOW, MEDIUM OR HIGH CHO	URINE				DIAGNOSIS
						NUMBER OF DAILY DETNS AVERAGED	VOLUME	REDUCING SUGAR		
			YEARS	KILOS			C.C.	PER CENT	GRAMS	
12-20	E-y.	♀	39	63	Medium	2	1110	0.079	0.88	Gastric subacidity, cystitis, endometritis
12-20	R-d.	♂	28	64	Medium	1	1000	0.113	1.13	Gastric hyperacidity, neurasthenia
4-21	S. B-n.	♂	36	73	Medium	2	1140	0.101	1.15	Gastric neurosis
1-21	E-e.	♀	19	59	Medium	2	705	0.086	0.61	Perimetritis, colitis, tonsillitis
5-21	J. B. T-d.	♀	44	47	Medium	1	1184	0.058	0.69	Menopause, anemia, menorrhagia
2-21	T-n.	♀	44	54	Medium	19	1895	0.037	0.70	Fibromyoma-uterus, anemia, neurasthenia
1-21	H-r.	♀	33	49	Medium	2	945	0.053	0.50	Cholecystitis, constipation, neurasthenia
12-21	J. B-s.	♂	41	—	Medium	5	2061	0.063	1.30	Hemolytic jaundice
12-20	C. S-d.	♂	54	63	Medium	2	1680	0.053	0.89	Chronic appendicitis, pericaecal adhesions
12-21	H. F-t.	♂	35	—	Medium	5	1437	0.082	1.18	Eczema
1-21	T. Van D-n.	♂	60	63	Medium	3	1560	0.124	1.93	Liver cirrhosis, intestinal stasis
10-20	L. H. M-r.	♀	41	66	Medium	1	1190	0.048	0.57	Middle ear vertigo
1-22	A. N-l.	♂	50	72	Medium	1	8800	0.0102	0.90	Optic atrophy,—question of brain tumor
4-21	T. B-e.	♂	28	54	Medium	2	570	0.130	0.74	Encephalitis lethargica
1-22	I. C-y.	♀	31	59	Low	5	914	0.158	1.44	Lactosuria, secondary anemia
1-22	B. T-y.	♀	11	39.5	Very low	4	391	0.089	0.35	Mild diabetes, (urine "sugar-free")
3-22	A. B-a.	♂	6	19.5	Very low	9	1315	0.039	0.51	Diabetes mellitus, (urine "sugar-free")
12-21	M. S-d.	♀	20	61	Very low	7	1150	0.049	0.57	Diabetes mellitus, (urine "sugar-free")
1-22	S. F-n.	♀	53	—	Very low	4	1185	0.053	0.63	Diabetes mellitus, (urine "sugar-free")
12-21	S. S-z.	♀	54	—	Very low	2	750	0.100	0.75	Diabetes mellitus, (urine "sugar-free")
12-21	V. L-n.	♀	59	77	Very low	2	1175	0.071	0.84	Diabetes mellitus, (urine "sugar-free")
11-21	S. S-l.	♂	61	53	Very low	10	1135	0.075	0.85	Arteriosclerosis, diabetes mellitus, (urine "sugar-free")
9-21	T. M-s.	♀	16	45	Medium	2	1530	0.061	0.93	Blood sugar of 0.157 per cent

*Low carbohydrate diet contains approximately 100 gm. carbohydrate daily, medium CHO diet about 250 to 300 gm., and high CHO diet over 300 gm. For children the amounts are smaller.

*Low carbohydrate diet contains approximately 100 gm. carbohydrate daily, medium CHO diet about [illegible] gm., and high CHO diet [illegible] gm. For children the amounts are smaller.

TABLE V—CONT'D

AVERAGE DAILY SUGAR EXCRETION IN NORMAL AND PATHOLOGICAL URINES

DATE, MONTH AND YEAR	CASE	SEX	AGE	WEIGHT	DIET,* LOW, MEDIUM OR HIGH CHO	URINE				DIAGNOSIS
						NUMBER OF DAILY DETNS AVERAGED	VOLUME	REDUCING SUGAR		
			YEARS	KILOS			C.C.	PER CENT	GRAMS	
3-21	P. C-r.	♂	17	68	Medium	3	1370	0.133	1.83	Diabetes, vascular hypertension
6-21	M. C-t.	♂	47	54 Lost 27 kilos in last year	Medium	3	1073	0.289	3.10	Cardiospasm, (one sister has diabetes)
5-21	L. N-l.	♀	45	68	Medium	5	1221	0.281	3.43	Chronic interstitial nephritis, history of diabetes
11-21	E. Mc C-y.	♀	25	48	Medium	3	910	0.585	5.32	Blood sugar of 0.217 per cent
1-22	J. C-k.	♀	58	—	Very low	1	575	3.100	17.85	Diabetes mellitus, (urine contains sugar)
						1	1200	0.101	0.87	(urine "sugar free")
1-22	M. A-s.	♂	45	68	Very low	1	1080	3.400	36.80	Diabetes mellitus, (urine contains sugar)
						1	782	0.100	0.78	(urine "sugar free")
11-21	S. N-r.	♂	43	61	Very low	1	1710	1.520	26.00	Diabetes mellitus
12-21	R. C-y.	♂	42	50	Very low	1	2160	1.500	32.4	Diabetes mellitus
3-22	S. L-y.	♂	9	23	Very low	1	2220	1.800	39.9	Diabetes mellitus
7-21	H. M-l.	♀	5.5	16	Low	1	235	0.049	0.12	Cardiac insufficiency, edema
7-21	J. D-a.	♀	3.8	12.5	Medium	4	275	0.047	0.13	Empyema—old
6-21	M. Mc G-h.	♀	2.5	13.5	Medium	1	105	0.182	0.19	Chronic arthritis
7-21	J. G-y.	♂	6	20	Medium	1	630	0.032	0.20	Foreign body in right bronchus
6-21	J. M-o.	♂	4.8	12	Medium	7	219	0.110	0.24	Psoas abscess
7-21	E. K-n.	♀	3	12	Medium	1	290	0.114	0.33	Erythema multiformis
6-21	S. B-d.	♂	5.7	18.8	Medium	8	390	0.090	0.35	Chronic interstitial nephritis
7-21	C. M-o.	♀	6	20	Medium	9	508	0.075	0.38	Chorea, endocarditis
4-21	O. H. S-s.	♂	7	—	Medium	5	1064	0.037	0.39	Asthma, constipation
3-21	J. P-s.	♂	9	23	Medium	6	373	0.115	0.43	Chronic parenchymatous nephritis

*Low carbohydrate diet contains approximately 100 gm. carbohydrate daily, medium CHO diet about 250 to 300 gm., and high CHO diet over 300 gm. For children the amounts are smaller.

fifty year old man suffering from optic atrophy, is interesting since it shows that even with the enormous excretion of urine in twenty-four hours,—8800 c.c.,—the amount of sugar excreted was normal, 0.90 gram, in a concentration of 0.0102 per cent.

The daily sugar excretion was studied in ten children suffering from various disorders, and an average range from 0.12 to 0.43 gm. was found. Smaller body weight and lower food intake may be the factors responsible for the lower excretion of sugar by children than by adults.

Increased sugar excretion in diabetes mellitus is a well-known phenom-

TABLE VI

THE EXCRETION OF FERMENTABLE AND NONFERMENTABLE SUGAR IN DIABETES

CASE	DATE	URINE							DIAGNOSIS
		VOLUME	REDUCING SUGAR						
			QUALITATIVE BENEDICT TEST	BEFORE FERMENTATION		AFTER FERMENTATION		NONFERMENTABLE SUGAR	
	1922	c.c.		PER CENT	GRAMS	PER CENT	GRAMS	PER CENT OF TOTAL	
B. T-y.	1/ 8-9	460	Sl. +	0.147	0.68	0.089	0.41	60.3	Mild diabetes
	1/ 9-10	460	Neg.	0.077	0.35	0.054	0.25	70.6	
S. F-n.	1/ 8-9	1400	Neg.	0.065	0.91	0.036	0.51	56.0	Diabetes mellitus
J. C-k.	1/ 6-7	900	+	0.260	2.34	0.087	0.78	33.3	Diabetes
	1/ 7-8	575	Strongly +	3.100	17.85	0.107	0.61	3.4	mellitus
	1/ 8-9	800	Sl. +	0.220	1.76	0.067	0.54	30.7	
	1/ 9-10	600	Sl. +	0.167	1.00	0.076	0.46	46.0	
	1/10-11	1200	Neg.	0.101	0.87	0.058	0.69	79.3	
M. A-s.	1/ 8-9	1080	Strongly +	3.400	36.80	0.098	1.07	2.9	Diabetes mellitus
R. C-y.	1/ 5-6	2400	Strongly +	1.500	36.00	0.034	0.82	2.3	Diabetes
	1/ 6-7	2480	Strongly +	2.000	49.60	0.038	0.95	1.9	mellitus
	1/ 7-8	2300	Strongly +	2.500	57.50	0.036	0.83	1.4	
	1/ 8-9	2550	Strongly +	3.000	76.50	0.037	0.95	1.2	
I. C-y.	1/ 5-6	800	+	0.196	1.57	0.196	1.57	100.0	Lactosuria,
	1/ 6-7	1300	+	0.156	2.03	0.105	1.36	67.0	12 weeks after
	1/ 7-8	850	+	0.230	1.95	0.104	0.88	45.0	parturition
	1/ 9-10	620	Sl. +	0.133	0.83	0.110	0.68	82.0	
	1/10-11	1000	Neg.	0.081	0.81	0.070	0.70	86.4	

enon. In our series in Table V we show the sugar excretion in a few diabetic patients. The range shown there is from 1.83 to 39.90 gm. reducing sugar daily, when the urine gives a positive reaction with Benedict's qualitative copper reagent. Of the diseases we have studied, diabetes mellitus is the only one in which we have noted sugar excretions markedly above the normal. The case of M. C-t. (Table V), a forty-seven year old man suffering from cardiospasm, is of interest. In the last year this patient lost 27 kilos in body weight. He has one sister who has diabetes. On June 9, 1921, he showed a fasting blood sugar of 0.145 per cent, and on June 17 a high normal blood sugar of 0.120 per cent, while for three days after June 17 he showed

an average daily sugar excretion of 3.10 gm. on a medium carbohydrate diet. The average sugar concentration in the urine was 0.289 per cent; these urine specimens gave positive Benedict qualitative tests.

Determinations of the sugar in the twenty-four hour urines of ten diabetic patients were made when the urines were "sugar-free," i.e., gave a negative result with Benedict's qualitative copper reagent. It is seen (Table V) that under these conditions the sugar output is practically normal, showing an average range from 0.35 to 0.93 gm. daily. This observation is in harmony with the findings of Felsher,[14] who showed that as long as the diet of diabetic patients contains a total glucose equivalent below a certain limit (which varies with the individual), the quantities of sugar excreted are not greater than those excreted by normal individuals. She showed also that after this limit is passed, further addition to the diet causes rapid and sudden increases in the sugar excretion.

In Table VI are shown the amounts of reducing sugar excreted by several diabetic patients, as determined before and after fermentation with yeast. It is seen that when the urine gave a negative reaction with Benedict's qualitative reagent, the percentage of nonfermentable reducing sugar was high, 56 to 79.3 per cent of the total reducing sugar. When there was only a slightly positive reaction to Benedict's reagent, the nonfermentable sugar was 30.7 to 60.3 per cent of the total sugar. On the other hand, when there was a strongly positive reaction to Benedict's reagent, the nonfermentable sugar represented only 1.2 to 3.4 per cent of the total, since by far the largest part of the reducing sugar excreted was glucose. The last case in Table VI was that of a woman, I. C-y., who had lactosuria. The excretion of lactose decreased until finally the urine was negative with Benedict's qualitative test. The percentages of nonfermentable sugar in this case are not unlike those observed in two normal men by Benedict, Osterberg and Neuwirth.[2]

SUMMARY

A comparative study of different methods for the determination of sugar in normal urine has been made. By the use of one of these methods, that of Benedict and Osterberg,[4] it was shown that there is an increase in the amount of reducing sugar excreted hourly after meals. It was found that a diet rich in carbohydrate increases the amount of sugar excreted over that on a low carbohydrate diet.

The daily excretion of reducing sugar in the urine was determined in 12 normal individuals and 140 hospital cases. In none of the diseases studied, except diabetes mellitus, is there any marked variation from the normal sugar excretion on ordinary diets. Moreover, in diabetes when by dietary regulation the patient is rendered "sugar-free," the daily amount of sugar excreted is practically normal.

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THE ETIOLOGY OF GALLSTONES*

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IN DISCUSSING a problem as old as the subject of gallstones, it is quite essential that one do so with caution and due modesty. It is a problem that has occupied the minds of scientific men for centuries because very early in the practice of medicine, physicians were called upon to treat this baffling ailment, and render succor to those so afflicted. The following communication is offered with the sincere hope that it may be of aid in reaching a more definite conclusion as to the etiology and alleviation of this very common disease.

Many theories and predisposing causes have been suggested to explain the formation of gallstones. A few of the important factors already suggested will be briefly reviewed before entering into the subject-matter of this paper.

It has been noted that gallstones are more common in women than in men, and that it is a disease confined largely to middle or advanced life. Likewise we know that it occurs more frequently in individuals of sedentary habits than in active persons engaged in hard physical labor.

Frerichs[1] suggested quite early that the essential factor for the formation of stones was a stasis or stagnation of the bile. Somewhat later the popular theory of the infectious origion of gallstones was advanced by Naunyn.[2] This theory was championed by such men as Gilbert, Girode, Dufort and Chiari.

Following the contribution of Naunyn, Cushing[3] advanced the theory that the essential conditions necessary for stone formation were stagnation of the bile or obstruction with the presence of infection. This theory is really a combination of the ideas of Frerich and Naunyn. An interesting and important contribution to the study of gallstones was later made by Kramer[4] who showed that filtered normal bile to which peptone broth was added and infection then introduced, underwent changes that were strikingly like those seen in pathologic gall bladders. He noted that under these conditions, cholesterin pigment and calcium salts settled out in the course of a few months and con-

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cretions of the same composition as gallstones formed. This work was subsequently confirmed by Baemeister.[5] Brockbank[6] then showed that bile obtained postmortem if admixed with desquamated bladder cell, on standing, showed a deposit of cholesterin. Sterile normal bile shows the same phenomenon after standing for prolonged periods of time. Aschoff[7] states that pure cholesterin stones are always obtained from bladders showing distention but never occur in chronically inflamed organs. He noted that the bile in these cases was frequently sterile. Pure cholesterin stones are very rare, as in the vast majority of cases, slight amounts of mucoid matter, pigment and salts, are present.

Adami[8] suggests that the probable steps in gallstone formation are:

1. Infection (B. coli, pyogenic cocci, B. typhosus).
2. Stagnation of bile.

As a result of this stagnation, an absorption of alkaline substances takes place with the production of an acid bile. This leads to a catarrh with an outpouring of mucus in which the pigments and salts are precipitated. He states that stones may be found anywhere in the biliary passages. In my studies up to the present time I have never encountered an acid bile and this explanation does not seem logical since we would expect in such cases to find gallstones composed largely of bile salts, since they are precipitated in an acid medium. Bile after prolonged stagnation and putrefaction undergoes an ammoniacal change and often becomes intensely alkaline in reaction.

The chief forms of stones encountered in clinical practice are those composed of cholesterin alone, or cholesterin and pigment and $CaCO_3$ (laminated gallstone) and the common gallstone which is made up largely of cholesterin and bilirubin calcium with small amounts of $CaCO_3$. The cholesterin gravel which consists of fine deposits of amorphous cholesterin is probably an early stage of stone formation.

Another form is described as the pure bilirubin calcium calculi or black bile gravel lying in a mucoid bile. These are soft stones embedded in mucus, containing calcium salts, bilirubin and a trace of cholesterin. This latter type, unfortunately, I have not encountered.

An extremely rare type is also described as the calcium carbonate stones. These are found chiefly in the herbivora but rarely ever in man.

To the above etiologic factors may be added the reduction or absence of bile salts in the bile. This may be brought about by infection, or hepatic insufficiency. Mayo-Robson[9] suggests this factor as an etiologic possibility. The following work deals largely with this latter change and suggests the various findings which bring about this disturbed bile salt secretion. The importance of the bile salts in the production of gallstones will be elaborated and an attempt made to correlate these findings with what takes place in the body in cholelithiasis.

While performing certain routine tests on bile obtained at operation, after cholecystostomy and at postmortems, I was struck with the striking dissimilarities in various specimens which to outward appearances were alike. It occurred to me that it would be of interest in connection with urinary

determinations on cases of cholelithiasis, to likewise at the same time determine the nature of the bile secreted by the liver. The cases here presented are all cases of gallstones. Cystic duct obstruction was usually present, while in a few cases there occurred also common duct obstruction with jaundice.

In all cases the urine was examined prior to examination, and the urine and bile daily examined for fourteen to sixteen days after operation. As previously reported,[10] all such cases show an increase in bile salts and pigment in the urine before operation. As a result of the obstruction to the

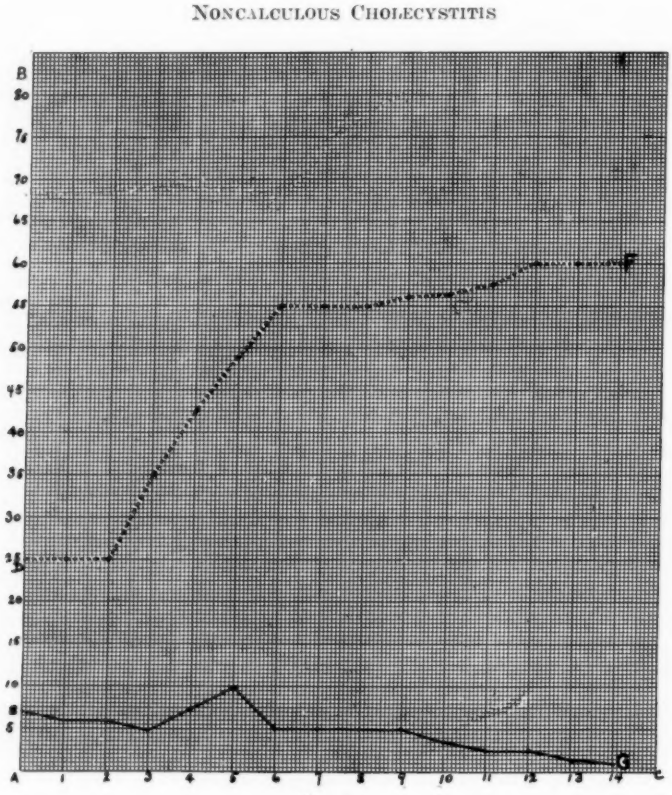

Fig. 1.

AB—Minims required to produce turbidity of test solutions.
AC—Number of days following operation.
EG—Bile at operation and after operation.
DF—Urine at operation and after operation.

normal outflow of bile, a retrograde movement of the bile into the liver undoubtedly occurs. This takes place if stasis alone is present but becomes more marked if actual obstruction also exists. Secondarily, absorption of bile into the lymph channels and blood takes place and increased excretion through the kidneys follows. In obstructive jaundice, both the pigment and bile salts appear in the urine, whereas in certain other conditions, notably cirrhosis of the liver, one element may appear in the urine without an appreciable increase of the other. Thus in certain cases one finds an enormous in-

crease in bile salts without the normal coloring matter of the bile, while in others the pigment predominates to the exclusion of bile salts. For this reason, it is obviously wrong to assume that unless bile pigment is present in the urine, there can be no increase in bile salts. Both should be tested for if a clear conception of the changes taking place is desired.

At operation the bile in each case was aseptically collected and tested for its various constituents. A striking feature of this bile was the diminution or absence of bile salts. Numerous specimens gave negative Hay's tests for bile salts and the Pettenkoffer and peptone reactions were likewise negative or very faint. Quantitative determinations of the bile salts present were made by treating the bile with absolute alcohol and then filtering it to remove the mucin or nucleoalbumin present. The filtrate was then tested with definite amounts of peptone and starch solutions and the amount necessary to produce a turbidity of these solutions determined. These were tested against known bile salt solutions and the bile salt content thus determined. The bile salt content of the bile was found to range in cases of cholelithiasis, from $\frac{1}{64}$ of 1 per cent to $\frac{1}{2}$ of 1 per cent.

Certain other cases relatively few in number, of catarrhal cholecystitis were seen in which the bile salt content of the bile was found to be low. The bile salt figures in certain of these cases ranged from $\frac{1}{10} - \frac{1}{20}$ of 1 per cent. These cases were treated as potential gallstone cases and responded well to cholecystostomy. Other cases have been observed where cholecystectomies were done where the bile salt content of the bile was found to be normal. These were noncalculous cases where the gall bladder was removed for other reasons. It is my belief that in such cases, if the gallbladder presents a healthy appearance, and the bile itself is chemically normal, a cholecystectomy is not indicated.

An organ such as the gall bladder possessing definite functions should not be removed, unless these functions are impaired or destroyed. It should be remembered that in most cases of gall bladder disease we are in reality dealing with hepatic insufficiency. Our problem is therefore, to so treat our cases that the liver again becomes a normally functioning organ. This point will be discussed later in this paper but it is mentioned here because of the present day tendency to remove gall bladders, as if they were functionless organs that the body can easily do without.

The bile obtained in gallstone cases where obstruction was present was usually dark green or black in color, of tarry consistency and relatively rich in mucin. The urine, if examined at this time, gave positive tests for bile salts and contained as large or larger percentages of bile salts than the bile itself.

Following operation, the bile and urine were daily collected and tested out as above described. After drainage of the gall bladder and removal of the stones, the urine tended to show daily decreases in bile salt, and the bile corresponding increases. Daily variations were noted and it was observed that the removal of the obstruction did not lead to immediate return to normal function on the part of the liver. Various grades of hepatic insufficiency

were noted and in two fatal cases the liver did not regain its normal functions even after operation. In these two cases the bile remained practically free of bile salts and greatly diminished as to quantity. The day prior to death only one dram was secreted through the tube in twenty-four hours. The urine in the meantime became scanty in amount, highly concentrated and contained bile salts and pigment in excessive quantities. In these two cases the urine and the bile could be scarcely differentiated by physical appearances.

In the other cases the urine reached normal limits in six to eight days and the bile in ten to fourteen days. About the fourth day after operation and for several days thereafter, an increase in urea occurred in the urine.

BILE SALT CONTENT OF URINE BEFORE AND AFTER OPERATION

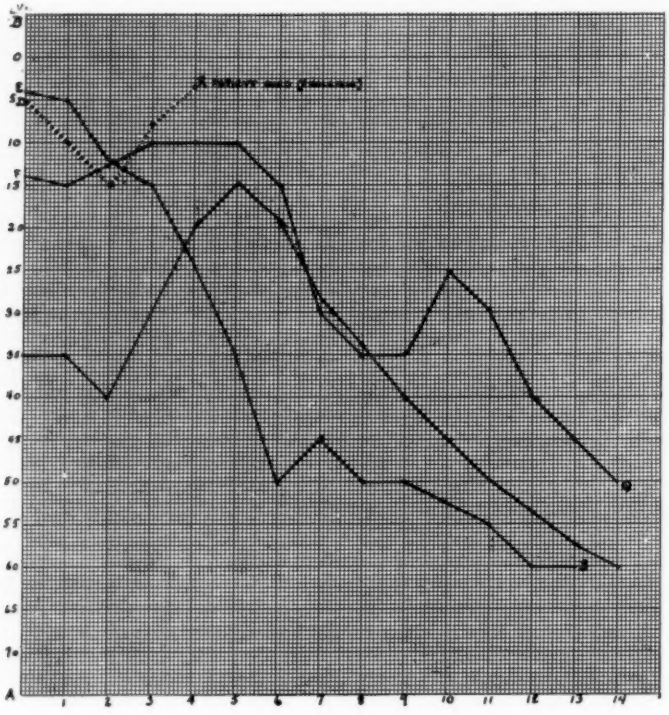

Fig. 2.

AB—Number of minims of urine required to produce turbidity of test solutions.
AC—Number of days following operation.
ES and FQ—Cases that recovered.
DR—Curve seen in fatal cases.

With the increase of urea there seemed to be a decrease in bile salts in the urine.

The accompanying charts graphically represent the changes above described. Both favorable and unfavorable cases are here depicted and the contrast between the two is quite striking. It should be noted also in connection with these observations on the bile that the urinary secretion seems to run almost parallel to the secretion of bile. Suppression of urine is a common complication in fatal cases of cholemia. These processes are not

merely coincidental but rather, the renal injury is dependent on biliary dysfunction with consequent toxemia. The bile, no longer finding access to the biliary channels, and intestine for its excretion, finds its way into the lymphatic and blood streams and the excretion of these toxic substances through the kidney produces a toxic nephritis with suppression of urine. Thus the mode of death is not particularly puzzling or difficult to explain. In reality we are dealing with primary hepatic insufficiency and a secondary nephritis of hepatic or biliary origin. In a subsequent publication the results of certain blood analyses will be reported by Dr. S. Tashiro and myself which further confirm the observations presented in this paper. These find-

BILE SALT CONTENT OF BILE AT OPERATION AND AFTER OPERATION

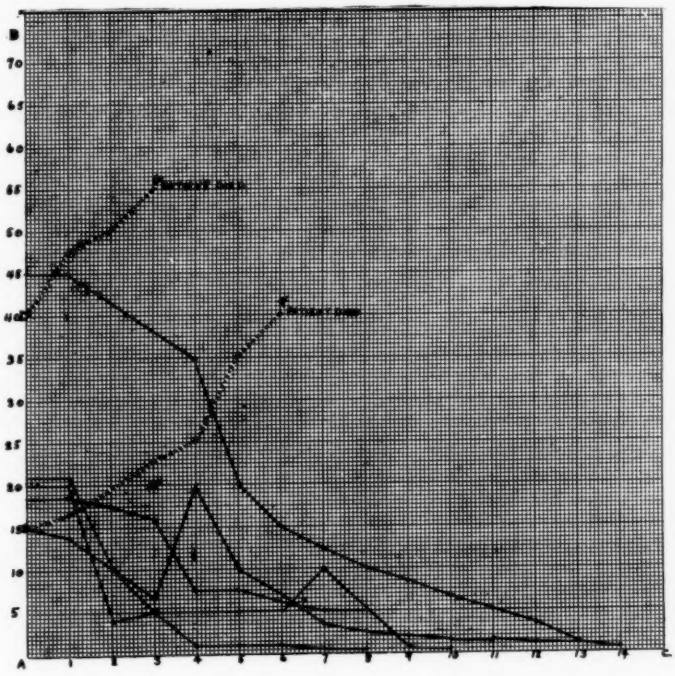

Fig. 3.

AC—Number of days following operation.
AB—Minims of bile (alcoholic filtrate) necessary to produce turbidity of test solutions.
DG and EF represent curves seen in fatal cases.

ings show that in all such cases as here described, there exists an increased bile salt content of the blood. Together with these findings it should be noted that we are also dealing with a cholesterinemia in all gallstone cases. An increased cholesterin blood content, together with a decrease in the bile salt content of the bile, provides an ideal soil for the formation of gallstones.

The findings above reported are of considerable importance in the elucidation of the formation of gallstones. It is known that the bile salts are the chief elements concerned in holding the cholesterin and lecithin of the bile in solution. Likewise it is known as stated previously, that cholesterin is the chief substance found in most gallstones. The occurrence of an in-

crease in cholesterol in the blood in cases where an increased bile salt content is found is probably in the nature of a protective mechanism. Matthews[11] states that cholesterol neutralizes the toxic action of hemolytic substances such as saponin, glucosides, and bile salts thus protecting the red blood corpuscles of the body from excessive destruction. It accomplishes this purpose by forming a weak molecular union with these substances. The hemolytic action of bile salts on red blood corpuscles is, in all probability, the result of a bile salt-protein chemical interaction and is similar to the effect of bile salts on the pneumococcus and in dissolving the tails of human spermatozoa. The nature of this chemical process will be discussed at length in a subsequent publication dealing with certain bile salt-colloid phenomena.

The occurrence of anemia, often severe in gallstone cases with obstructive jaundice, has been repeatedly observed and is, in all probability, due to excessive blood destruction resulting from the hemolytic action of bile salts.

Another point of interest while discussing cholesterol is that it forms one of the chief constituents found in the deposits on the walls of arteries. The underlying basis for this cholesterin deposit is at the present time conjectural.

In cases of cholelithiasis then, it would seem that an intimate interrelation exists between the cholesterol content of the blood and the bile salt index there found. Further, the relationship between the deposit of the cholesterol in the bile and the bile salt content of this substance is a most important one.

From theoretical grounds, it would appear logical that any condition leading to the removal of the bile salts from the bile should favor the settling out of these substances from the bile.

Bile obtained at operation from gallstone cases, after having been examined chemically, was then placed in test tubes, sealed, and allowed to stand for varying periods of time. It was thought that if the chemical conditions for stone formation were present in such bile, a settling out of cholesterin should occur. Normal bile was also obtained and the different specimens so secured were observed. It was found that those specimens of bile which were deficient in bile salt contained a deposit of cholesterin in the bottom of the tube, often in a very short time. All such specimens showed this change within two to three weeks, while in some it appeared in three to four days. Normal bile or bile rich in bile salts, remained free from cholesterin deposits. This simple experiment was formed with approximately seventy-five specimens of bile obtained at and after operation and the same general changes were observed as described. It could be accurately predicted, after discovering the bile salt content of the specimen, which tubes would show cholesterin deposits and which would not. Curiously enough, in a number of tubes, the cholesterin settled out in concentric layers, alternating with layers of pigment, thus bearing a striking similarity to true gallstones.

As we know that cholesterin is the chief element found in gallstones, a plausible explanation of stone formation here is offered. On this hypothesis

stones need not necessarily be formed in the gall bladder alone, but may be found anywhere in the biliary tract. That they should collect in the gall bladder primarily is to be expected. In all probability they are formed elsewhere as well and are washed into the gall bladder and there retained. The probable steps in the formation of stones are about as follows:

First: There occurs a bile stasis; this is probably most frequently brought about through infection. It is entirely conceivable, however, that stone formation may occur independently of infection. Any factor or factors responsible for bile stasis may be looked upon as predisposing causes.

Second: As a result of the stasis produced, a back-damming into the liver occurs.

Third: Consequent to this change a certain degree of hepatitis and hepatic insufficiency occurs.

Fourth: As a result of this change in physical conditions and impairment of liver function the bile salts are not excreted into the bile as they normally are.

Fifth: If this condition is merely temporary, a condition of "biliousness" probably exists which is relieved by cholagogue cathartics such as calomel and $MgSO_4$.

Sixth: If the condition extends over a considerable period of time the cholesterol of the bile is no longer held in solution and tends to settle out. It tends to collect around some foreign nidus, dragging down a certain amount of pigment and mucin with it. The development of these concretions tends to further increase the stasis present, and, if not relieved by drainage, must inevitably lead to further stone formation. If actual obstruction occurs, a vicious circle is established which can only be corrected by operative intervention. If the process has gone on for a long period, the damage to the liver may be such that only temporary relief will be obtained. This, I believe, accounts for the numerous symptoms which sometimes persist after operation. We must remember that in reality we are dealing with hepatic disease even more than with disease of the gall bladder. If the liver can be restored to a normal state, we have obtained a real cure.

To briefly summarize then, it would seem that in cases of gallstones and obstructive jaundice, a disturbance in bile salt secretion and excretion occurs.

This manifests itself by an increase in bile salts in the urine and blood and a decrease in the bile salt content of the bile.

As a result of the deficiency in the bile salt content of the bile, cholesterin tends to settle out of solution. If the condition is not early remedied, gallstones form the end result of this physico-chemical disturbance. Further the increase in bile salts in the blood leads to toxic changes in other organs, notably the kidneys and heart, and also profound constitutional disturbances of a toxemic nature. In all cases of cholemia, hepatic insufficiency is present.

Following operation, the urine becomes normal usually in six to eight days while the bile reaches normal limits in ten to fourteen days.

Finally, cases of this character should be so treated as to restore the bile, urine and blood to their normal physico-chemical state.

In conclusion I wish to express my very great thanks to Dr. Shiro Tashiro for his unselfish and valuable aid, and to Drs. A. P. Matthews, and Martin H. Fischer, for their many words of counsel and advice.

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CRESYLECHT VIOLET, A RARE DYE*

Observations Which Suggest That It May Be of Value in Pathological Work

By B. G. R. Williams, M.D., Paris, Ill.

THIS preliminary report is based upon work begun about 1915 and continued as well as possible during the past seven years with recesses due to difficulty in securing material consequent to the World War. Most of the work was done in my laboratory, but some of it has been carried out at St. Anthony's Hospital (Terre Haute, Indiana), where all excisions are worked up routinely.

Credit for assistance in the work is gladly given to Dr. H. J. Conn, Chairman Committee on Standardization of Stains, National Research Council and to the various American dye manufacturers and jobbers who have furnished materials and who will be mentioned later in this connection.

Cresyl violet (cresylecht violet, not crystal violet) is a rare dye. Apparently it has been used but little or not at all in this country. There is but little in the literature on the subject; and this is foreign. The method of preparing cresyl violet was developed by Bender in 1892. It is a derivative of cresyl blue and is probably (?) prepared from cresyl blue by sulphonization or alkalization to obtain the proper violet shade. Although there are (or have been) a number of stains marketed under the name of cresyl violet, they all have certain properties in common; and cresyl violet is not identical with cresyl blue, as some manufacturers have claimed it to be.

Cresylecht violet is not listed in the main table of Schultz' Farbstofftabellen[1] but it is mentioned in the index of this book where it is referred to presumably as a new or comparatively unimportant dye. In this index three

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shades of cresylecht violet are mentioned, denoted respectively as B, BB and 2RB. It is stated that this dye was manufactured by the Farbwerk Mühlheim of Frankfort a. Main, formerly A. Leonhardt and Company. As will be shown below, it has been difficult to secure under the name, "Cresyl violet or cresylecht violet, Gruebler" any two products with identical properties.

My attention was directed to cresyl violet perhaps ten years ago, but later by the descriptions of Krause[2] who secured beautiful results with tissue sections in normal histology. However the product was mentioned by him as cresyl violet, RB. It was described by him as an oxazin, coming into commerce as a bluish-violet powder, dissolving with the same color production and was claimed by him to give beautiful metachromasia in fresh tissues and formalin fixed tissues. I have never been able to learn of its use in pathological work,* although cresyl violets have been referred to in connection with normal histological sections. Herxheimer[3] used Kresylecht-violett for staining sections of skin; and Fink[4] used one of these stains for similar work. Also Bielschowsky and Plien[5] employed concentrated cresyl violet, cold for twenty-four hours, differentiated by alcohol, for tigroid substance.

WHERE USED IN TISSUE WORK

In my hands the stain has proved of promise in two kinds of pathological work; viz.,

1. In the so-called biopsy, where unfixed tissues are stained for purpose of rapid diagnosis. This method has been used in certain hospitals with a view to securing a diagnosis during an operation, that the surgeon may judge as to the probable extent of the disease or the best method for completing the operation. Here for several years cresyl violet has been giving me better results than I can secure with other stains used for this purpose. Perhaps the staining is not so rapid but there is better metachromasia and the pictures are clearer. Other stains which are being used for this work are thionin, Wilson's stain, Goodpasture's stain, Thibault's stain, Terry's stain, etc., most of them alkaline methylene blue solutions. Roughly the method is to throw several of the sections into a watch glass of the stain (dissolved to concentration or strong solution in cold distilled water and filtered) and removing after a minute or so and examining in water after a brief washing in water. The sections should be fairly thin and should not overlap in the stain. As stated, the method does not seem to be quite so prompt as with the various alkaline methylene blues, but the cells are stained and diagnoses are possible which are hopeless where only tissue schemes are made out. Metachromasia is good, the nuclei (chromatin) taking a deep blue or violet; the cytoplasm, some other shade of blue (depending upon the cell-type); the collagen fibrils taking a deep red; the fibroglia fibrils, pink; fat droplets often a yellow or orange and the erythrocytes appearing as green discs, etc.

2. But a more promising class of work has been that which, for want of a better name, I have called "Diagnostic Surgical Tissue Work" or fairly rapid work with frozen-formalin fixed tissues where a metachromatic stain

*Since the above was written, I have learned that Warthin has used Kresyl-echt-violett in pathological work, particularly as a specific stain for amyloid and mucin. He has used it for the last ten years. To each gram of the dry stain, he uses 80 c.c. carbolic acid and 20 c.c. alcohol.

TABLE OF RESULTS

	NO. 1197	ORIGINAL BATCH (GRUEBLER)	2ND. SAMPLE (GRUEBLER)
Appearance	Dark blue.	Dark violet.	Dark violet.
Soluble	In water.	In water.	In water.
Stability of Solutions	After 6 weeks is stable in Aq. dest.	Stable in 10% formalin but not in water.	Not stable, even in formalin. Blue first lost, leaving a red fluorescent solution.
Color of Solution	Deep blue with a violet shade in strong transmitted light.	Deep violet.	Violet.
Color of Wash Waters	Blue.	Deep red and fluorescent.	Red. Very fluorescent.
Fluorescence	None.	Marked.	Very marked.
Unfixed Tissues	Good results in aqueous solution.	Good results in formalin solution.	Fails to stain in 2 minutes.
Fixed Tissues	Good results in aqueous solution.	Good results in aqueous or formalin solution.	In 2 minutes cytoplasm becomes pink. Eventually the nuclei take a light blue.

Résumé,—

1. The results with this American cresylecht violet are good. It is possible that this product is similar, identical or better than that described by Krause as cresyl violet RB.
2. The two samples of Gruebler cresylecht violet, are not identical.
3. These dyes seem to consist of two separate parts or properties, a blue and a red. Those which are mainly blue are more stable. They all show metachromasia. Where there is more red the violet is more marked than the blue reaction. Past a certain point the increase of red property gives a very unstable solution and one which does not satisfactorily stain the nuclei.
4. Concerning No. 1197.
   a. It is much superior to Second Sample (Gruebler).
   b. It is much more stable than Original Batch (Gruebler).
   c. It stains more rapidly and more intensely the nuclei than does either other sample.
   d. It stains materials which are not stained by the Gruebler samples, particularly the fat.
   e. It does not show the "violet metachromasia" of Original Batch (Gruebler), but it shows a sharp metachromasia and one which is satisfactory for pathological work. By adding to its red properties the "violet metachromasia" might possibly be realized. However this would doubtless be at the expense of its keeping properties in solution and with loss to rapidity of chromatin staining. I would recommend that no changes be made in the dye at present.
5. I would suggest that samples of No. 1197 be tried out by tissue pathologists with unfixed tissues and those fixed by formalin. I am securing best results with frozen sections (not too thick) and to avoid as much as possible overlapping of sections. I am using no celloidin sections. Our staining time varies with the particular tissue, the fixation and the kind of examination desired. It is usually 2—5 minutes. Sections cannot be mounted in balsam. Levulose does not give good mounts. I examine in water after washing in water. Any particular section may be de-stained by alcohol and a special method of staining used. At present I am using a strong or concentrated solution of the dye in cold, distilled water (filtered).

saves time and where many sections can be quickly handled and examined in water under a long rectangular cover glass, often avoiding double-staining, mounting, etc., etc. Or in case special double-staining is wished in any particular section it may be rapidly destained in alcohol and stained as wished for a mount. Roughly I have figured that by this method a dozen sections can be studied in the same time that the more elaborate methods will give one or two. Moreover such a method is of particular value where the diagnosis is fairly easy, where the interested parties are impatient for a diagnosis and where sections should be examined from a number of portions of the neoplasm. It often shows how great the variation of structure in several portions of the specimen, a point missed by the man who glances but one or two sections.

EARLY WORK WITH CRESYL VIOLET

After the armistice I found that cresyl violet of foreign make (and this was the only make) was not upon the domestic market, nor could it be secured very easily by importing. I learned this after about thirty-five letters to jobbers, manufacturers, importers, etc.

About twelve months ago, a cresyl violet of American make was put upon the market. This product was tested in my laboratory and found to be insoluble in water. Moreover, as regarded staining properties, it failed to resemble cresyl violet.

Somewhat later I was persuaded that cresyl blue was after all identical with cresyl violet; but investigation proved that it was not. Other substitutes were recommended and some of them tried out; but all failed to give results even approaching those of cresyl violet.

Then Dr. Conn was able to secure for me two samples of cresyl violet which have proved of great value in this work. One of these was some Gruebler cresylecht violet furnished me through the courtesy of the Will Corporation and the other was a sample of American cresylecht violet prepared by the National Aniline & Chemical Company of New York. The results of my investigation of the former showed great variation in the properties of Gruebler cresylecht violets, while study of the latter gave me a material which yielded better results than any of the foreign cresyl violets I have examined. Fortunately I still had on hand some of the Gruebler product secured before America's entry into the World War. Accordingly experiments with the three dyes were set up alongside and the results reported.[6]

In this report, No. 1197 refers to the cresylecht violet supplied by the National Aniline & Chemical Company. By original Batch (Gruebler) is meant the Gruebler stain secured before 1917; and by Second Sample (Gruebler) is meant the Gruebler stain furnished me by the Will Corporation, this last May.

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A NOTE ON THE DETERMINATION OF UREA IN BLOOD BY THE FOLIN AND WU METHOD. A MODIFIED APPARATUS*

By George G. Boggs and W. S. McEllroy, Pittsburgh, Pa.

IN THE determination of urea in blood by the Folin and Wu[1] method we have encountered difficulty in the distillation of the ammonia. The acid in the receiver may be drawn back into the distilling tube because of variations in pressure. This does not occur often when the determinations are conducted in a perfectly still room or when the burner is protected from drafts. However, it is not always possible to obtain these favorable conditions in a hospital laboratory or in a student laboratory. During the past two years our students have had considerable difficulty with the method on this account.

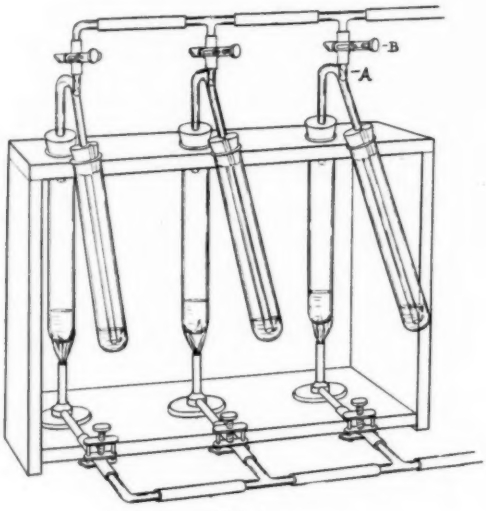

Fig. 1.

Watson and White[2] modified the delivery tube to prevent liquid being carried over as froth into the receiver. This does not overcome the difficulty we have encountered. Excessive frothing is moreover usually due to traces of protein in the filtrate because of incomplete precipitation or to faulty preparation of the enzyme extract.

By passing a slow current of air into the delivery tube so that a slight positive pressure is maintained during the distillation we have found that drawing back of the distillate is prevented. The arrangement employed is shown in Fig. 1. The air is introduced through the side tube A. After

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adding the borax to the contents of the distilling tube the rubber stopper carrying the delivery tube and receiver is firmly inserted into the distilling tube. The air current is started and so regulated by the screw clip B that about one bubble per second issues from the end of the tube in the receiver. The flame is then applied and the distillation conducted in the usual way. A number of determinations may be run simultaneously as shown in the diagram. In the absence of an air line in the laboratory the small volume of air required for a number of analyses may be supplied by air displacement from a large bottle by means of a water siphon.

It is important that the air current enter the connecting tube below the bend as shown in the diagram. If the air current is introduced at or before the bend, difficulty is encountered in driving all of the ammonia over into the receiver. This is probably due to condensation of the vapor in the tube by the entrance of the cool air.

We have found that no ammonia is lost even when a moderately fast air current is used, although this does not promote faster distillation and therefore a very slow current is best. No ammonia is lost if the distillation is prolonged several minutes. If the boiling is too vigorous and prolonged, however, a slight turbidity may appear in the distillate which interferes somewhat with the color comparison after Nesslerizaton.

We have tried the usual aeration combined with the steam distillation and have obtained good results but the above arrangement is preferable.

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TECHNICAL PRINCIPLES IN RADIAL TRANSMISSION SPHYGMOGRAPHY*

By H. M. Korns, M.D., and E. J. Warnick, Cleveland, Ohio

THOSE who have worked with radial transmission sphymographs are in position to appreciate the fact that the human forearm, with its varying size, shape, and consistency, offers unsatisfactory support for all such instruments. It is almost impossible to strap the base of the instrument to the

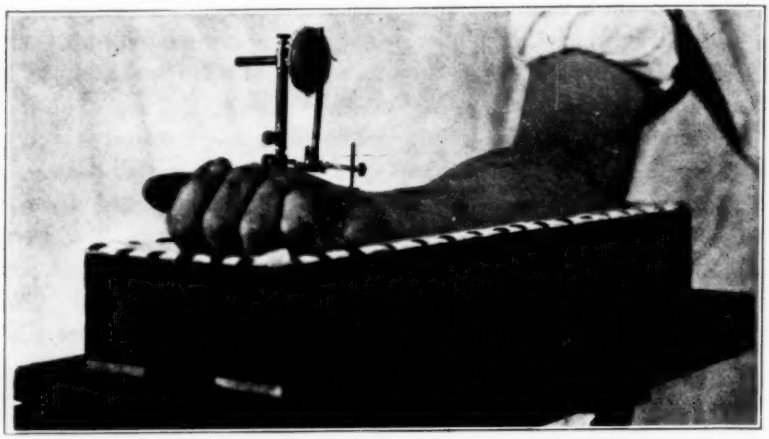

Fig. 1.

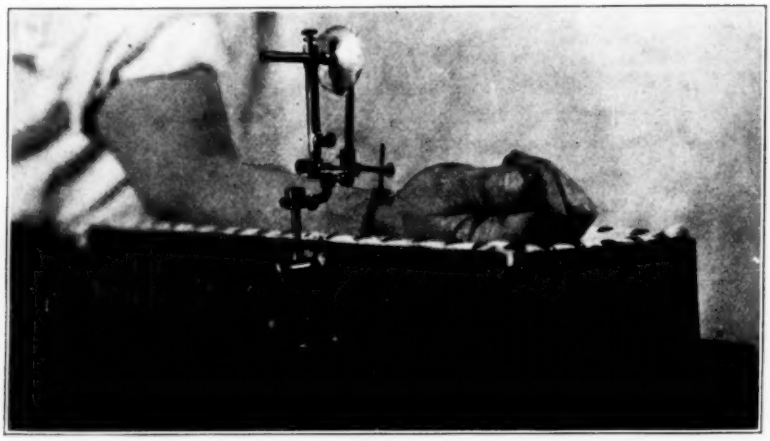

Fig. 2.

arm firmly enough to insure transmission through the sphygmograph lever of all the motion imparted by the radial pulse. Some of this motion is invariably lost through movement of the entire instrument upon the soft tissues of the arm. Furthermore, in order to bring the radial artery into bold relief it is necessary that the forearm be supported in the supine position in such a

*From the Medical Clinic of Lakeside Hospital.
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way that the third metacarpal bone forms with the dorsal surface of the wrist an angle of approximately 155 degrees. One cannot expect the subject to maintain this position, for it is essential to his comfort and to good technic that all the muscles of the forearm and hand be maintained in a state of complete relaxation; on the other hand it is difficult to provide suitable extemporaneous support.

To surmount these difficulties we have cast a plaster of paris bed for the dorsum of the forearm, using a moderately large subject as a model. Although this bed is much too large for a thin arm, its essential feature, namely, preservation in a comfortable position of the proper angle between the wrist and third metacarpal bone, is universally applicable. The plaster bed is lined with soft material, and may be additionally padded as required. It is necessary to prepare separate plaster beds for the right and left arms in order that tracings may be made from either radial artery. Absolutely rigid

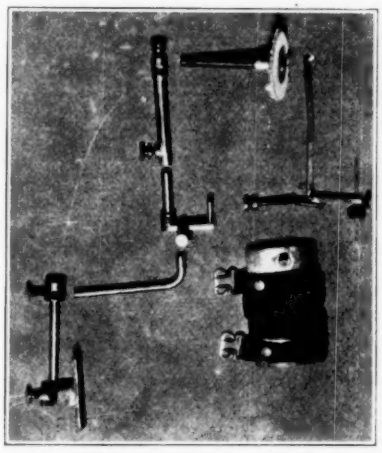

Fig. 3.

support for the sphygmograph is furnished by mounting it on the side of the box which encloses the plaster cast. By this means the wrist is caught firmly between the underlying support and the button resting upon the radial artery, an arrangement which affords ideal technical control. A distinctive feature of the instrument is the provision of ample means for adjustment, so that the optimal application of the button may be easily and quickly secured. The details of the arrangement are shown in the accompanying figures. When the tambour is connected in the usual way to an optical recording capsule the tracings conform in every respect to the requirements for accuracy. The sphygmograph itself is a modified form of that devised by Wiggers and Baker.* It is interchangeable with the median arm plate, as shown in Fig. 3. Because of the fact that the sphygmograph does not move with the arm, it is necessary, in order to secure an undistorted record, to prevent the slight movements of the arm which normally take place with respiratory excursion of the thorax.

*Wiggers and Baker: Am. Jour. Physiol., 1922, lix, 454.

REPORT OF A CASE OF CHRONIC HYDROCYANIC ACID POISONING*

By Jacob Rosenbloom, M.D., Ph.D., Pittsburgh, Pa.

CASES of industrial and chronic cyanogen poisoning are very rare. Weyl[1] states that he could find no case in any of the German factory inspectors' reports for twenty years prior to 1897, nor in some twenty-five volumes of foreign factory inspectors' reports. Rambousek[2] has found practically the same in a search of the modern literature. He reports two cases of cyanogen poisoning. In one case, the symptoms of vomiting, nausea, palpitation and fatigue developed after two years in a man working in the silver electroplating of copper plates where the plates were dipped in a solution of silver cyanide. The other case is one of acute poisoning of industrial nature.

The poisonous action of cyanogen and its compounds depends upon their power of absorption of oxygen from the blood with the result that the venous blood retains the color of arterial blood. This results in a tissue suffocation. At the same time they have an exciting and then a paralyzing effect upon the nervous system.

Most of the cases of chronic poisoning result from inhalation and if large quantities have been inhaled, death occurs almost instantly. In slight cases of poisoning the patient feels a sensation of irritation in the throat, giddiness, sickness, and dyspnea. A form of chronic hydrocyanic acid poisoning has been described in workers manipulating prussic acid and cyanids, with the following symptoms.

The workers complain of oppression of the chest, throat irritation, giddiness, difficulty in breathing, palpitation, hebetude, exhaustion, nausea and vomiting. Karitschoner[3] made some observations on patients who were made to breathe at intervals during many weeks, prussic acid vapor, with the idea that such a treatment would destroy the tubercle bacilli. One-fourth of those treated this way suffered from redness of the pharynx, salivation, headache, nausea, vomiting, slow pulse and albuminuria.

Cases of chronic poisoning have been described in photographers and those engaged in cyaniding gold ores and in galvano plating. Reed[15] has described cases of chronic poisoning from hydrocyanic acid and also chronic poisoning from cyanogen chloride.[16]

On account of the rareness of this condition, I feel the following case should be reported.

Mr. S., chemist, has been working for the past nine months at a chemical problem in which he uses a great deal of hydrocyanic acid. No change was noticed in his health during the first eight months. For the past month, however, he has no energy and vomits every meal, shortly after its ingestion, and he notices that his left eye twitches constantly.

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A complete physical examination shows normal organs. The pupils are slow in reacting to light and to accommodation, otherwise the neurological examination is negative. The urine is normal. The blood examination showed the following:

Hbg., 70; R.B.C., 3,500,000; W.B.C., 10,000; Diff. Normal.

It can be noted that there is present a moderate secondary anemia.

I feel that his symptoms and the secondary anemia are the earliest symptoms of chronic cyanide poisoning, on account of the fact that after one week's absence from the laboratory, the symptoms disappeared.

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A FATAL CASE OF BACILLUS ACIDI LACTICI MENINGITIS WITH AUTOPSY FINDINGS AND REVIEW OF BACILLUS MUCOSUS INFECTION*

By Henry M. Ray, M.D., Pittsburgh, Pa.

WHILE infection with members of the group of bacillus mucosus capsulatus (Friedlander) with isolation of the organism from the blood is relatively of rare occurrence, investigation of the literature fails to reveal a single case in which the bacillus acidi lactici has been isolated from the spinal fluid. In the case about to be reported, the bacillus acidi lactici was isolated twice each from the blood and spinal fluid during life and also from the brain, spleen, and liver after death.

In addition to the more commonly reported cases of bacillus mucosus pneumonia and septicemia, the organism is mentioned as being the exciting cause in epididymitis, purpura, multiple neuritis, suppurative cholecystitis, liver abscess, otitis media, angina and croup. Two cases of suppurative meningitis attributed to infection with this group are reported by Jassniger, 1901, and Sheib, 1900, the former a Friedlander diplobacillus infection, and the latter an infection with bacillus lactis aerogenes. It is to be noted that in the last two cases, the bacteriologic evidence is inconclusive since the examinations are incomplete and the reactions on the various carbohydrates are not recorded as seems indeed to be the case with many of the other reports of infection with the bacillus mucosus group. The incompleteness of methods employed, especially the failure to plant the organism on all the carbohydrates is largely responsible for the difficulty which apparently still exists in establishing a suitable classification of the members of this group. A practical classification based on the fermentation of certain carbohydrates and first recommended by Perkins has been adopted by many workers and is as follows: (1) The bacillus lactis aerogenes (Escherich) which possesses the most marked power of fermentation, producing acid and large amounts of gas in dextrose, lactose and saccharose broth. This member is probably the one most frequently observed. (2) The bacillus acidi lactici (the bacillus duodenale of Forde) possesses fermentation powers in a lesser degree and produces acid and gas in dextrose and lactose broth, but fails to attack saccharose, inosite, raffinose, zylose or inulin. The amount of gas production is much less than that produced by the bacillus lactis aerogenes. (3) The bacillus pneumoniae (Friedlander) ferments dextrose and saccharose to acid with a small quantity of gas production, but fails to ferment lactose. The discrepancies reported by bacteriologists in the reactions of this group to the

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carbohydrates are in all likelihood due to the fact that in many instances impure carbohydrates are used and in addition, the failure to recognize the fact that organisms occasionally lose their original fermentative powers as a result of changes in environment or other unfavorable conditions.

Jassniger's case occurred in a 16-year-old apprentice who suddenly became ill with severe chills, passing into a state of stupor and developing within a few days rigidity of the neck, hyperesthesia, irregular pupils and a definite Kernig with incontinence of urine and feces. Temperature rose above 39° C., with irregular remissions. Death occurred on the 7th day and the autopsy revealed a purulent cerebrospinal meningitis. Direct smears showed many intra- and extracellular capsulated bacilli with rounded ends. Cultures on agar, broth and gelatin produced a similar organism which was nonmotile and gram negative. A rabbit survived an intraperitoneal inoculation with ½ c.c. of broth culture while a mouse succumbed within 12 hours. No fermentation reactions on the carbohydrates are recorded and the author concludes that the organism was the diplobacillus pneumoniae of Friedlander.

Scheib's case was that of an 8-day-old girl, one of a pair of twins. The child died without showing any signs or symptoms of organic disease and the clinical diagnosis was "debilitas vitae." Postmortem examination, which was performed 8 days after death revealed a suppurative cerebral meningitis with distention of the tympanic cavities with pus. There were no other significant changes. The author gives a brief morphological description and after stating that the usual media were inoculated, he concludes that "taken in all, the evidence presented proves that the bacterium lactis aerogenes was present in the corpse." Here again no fermentation reactions on the various carbohydrates were attempted and considering the fact that there were no symptoms or signs of organic disease and that the postmortem was performed 8 days after death, it is highly improbable that there was an antemortem bacillus aerogenes lactis infection at all.

The case occurring in this hospital was that of a white American 55 years old, admitted to the medical service of Dr. C. L. Palmer, February 15, 1922, with the statement that 5 weeks previously he began to experience pain in the chest, cough, difficulty in breathing, severe headache and impaired appetite. The family history offered nothing of importance and the previous history stated that there had been the usual diseases of childhood and about 10 years ago, a fractured jaw.

The physical examination showed a well nourished middle-aged man apparently in a stuporous state and responding slowly to questions. Pupils were contracted and equal and there was a distinct arcus senilis. Examination of the chest revealed a few scattered moist râles with impairment of resonance over both bases. Heart sounds were distant, free from adventitious sounds and there was no apparent enlargement of the precordial area. The abdomen was scaphoid and tympanitic. The reflexes could not be elicited. The temperature on admission was 102° F. and varied between 99° and 104° with irregular evening remissions. The pulse was full and soft and varied between 92 and 134. Respirations at first averaged 24 to the minute,

increasing during the last two weeks to 42. Blood pressure was 196 systolic and 100 diastolic.

The course of the disease while in the hospital was characterized by marked confusion with severe headache alternating with periods of dullness. There developed definite rigidity of the neck with slight left-sided hemiplegia. Roentgenologic examination revealed marked density of the upper two-thirds of the right lung with intervening aerated spaces suggesting bronchopneumonia. The blood count showed hemoglobin 65 per cent, red blood cells 3,600,000, white cells 18,200 and 76 per cent polynuclear neutrophiles. Urinalysis revealed a trace of albumin and an occasional hyaline cast. The widal was negative in all dilutions. Blood Wassermann was negative to both crude and cholesterinized antigens and the blood chemical determinations were nonprotein nitrogen 51 mgs., urea 32, creatinin 0.9, uric acid 2.1, and sugar 120.

Blood cultures on various media taken February 25, and March 1, revealed in both instances numerous colonies of gram negative bacilli showing the interesting characteristics soon to be described and corresponding to the bacillus acidi lactici. A blood culture repeated 20 days later remained sterile after 72 hours. The spinal fluid, on two occasions (February 27 and March 7) yielded upon culture the identical organism. Both specimens of spinal fluid were a distinct canary yellow, globulin two-plus, and reduced Fehling's solution. Cell count, February 27, showed 108 with 92 per cent polynuclear leucocytes and March 7, 80 with 91 per cent polys. The colloidal gold curve of both fluids was distinctly of the meningitis type. The direct smears from the centrifugalized sediment revealed many gram negative bacilli which exhibited distinct capsules when stained by the Hiss, Rosenow and Nicolle methods.

The cultures recovered twice from the blood and spinal fluid revealed an organism which was a gram negative, nonmotile, nonspore bearing bacillus exhibiting a distinct capsule. The colonies on the blood plates appeared as slightly transparent, bluish white, rounded bodies with irregular outline and showing under the high power a delicate network of furrows. The bacilli averaged 1 to 2μ in length by .5 to .7μ in diameter and the ends were somewhat rounded. There were no irregularities in staining. Transplants into carbohydrates produced acid and gas in dextrose, maltose, mannite, arabinose, rhamnose, sorbitol, dulcitol, mannose, lactose and dextrin. It did not produce acid or gas in saccharose, innosite, raffinose, xylose and inulin. It produced acid on litmus milk and gave a positive indol reaction. Gelatin was not liquefied and after four days the extension of the growth along the line of the stab presented the typical nail-like appearance. Growth was luxuriant in practically all media and on agar it formed a thick white viscous layer. Milk was coagulated and smears from the milk culture exhibited the capsules most beautifully. Broth was diffusely cloudy after 24 hours and on the surface a viscous pellicle formed, making a ring around the tube; on further incubation the pellicle fell to the bottom but the medium remained cloudy.

A guinea pig inoculated intraperitoneally with ½ c.c. of a 24-hour broth

culture died within 4 days and showed upon examination a sanguino-fibrinous peritonitis with bronchopneumonia. The organism was recovered from practically every organ in the body.

Intravenous inoculation of 1 c.c. of a 24-hour broth culture into the marginal vessel of a rabbit resulted in death within 48 hours. The organism was demonstrable in all the viscera and in the brain. The morbid anatomy corresponded to that of any hemorrhagic septicemia.

The patient died after a 28-day stay in the hospital and the autopsy was performed 1 hour after death with the following findings:

Anatomic Diagnosis.—General. The bacillus acidi lactici bacteriemia. *Head.*—Acute and subacute fibrino-purulent meningitis; marked pial edema; multiple disseminated petechial hemorrhages of the cerebrum, cerebellum, pons and medulla; sclerosis of cerebral arteries. *Mediastinum.*—Acute and subacute mediastinitis with hemorrhagic infiltration; subacute adhesive pleuropericarditis; acute and subacute serofibrinous pleuritis. *Lungs.*—Unresolved lobular pneumonia; pulmonary congestion and edema; anthracosis. *Heart.*—Hydropericardium; fatty infiltration of the epicardium and myocardium; multiple petechial epicardial hemorrhages; cardiac dilatation with relative insufficiency of the valve orifices. *Peritoneal Cavity.*—Encapsulated retroperitoneal abscess. *Liver.*—Fatty cirrhosis. *Spleen.*—Acute splenic tumor with multiple intracapsular hemorrhages. *Pancreas.*—Sclerosis. *Adrenals.*—Cloudy swelling with congestion. *Kidneys.*—Congestion. *Prostate.*—Involution with sclerosis.

Résumé of Protocol. The body is that of a well developed and obese white male of good musculature and nutrition, weighing 190 pounds and measuring 68 inches in length. Right pupil is irregular and dilated and the left contracted. Skin of the chest, abdomen, lower extremities and in fact the mucosa of the lips are studded with innumerable pin-point to pea-sized petechiae which persist in spite of pressure. Abdomen is distended and tympanitic. The ankles pit on pressure.

In the right lower abdomen, entirely walled off, and retroperitoneal is an abscess cavity the size of an adult fist and bounded by the lumbar vertebrae, the ileo-psoas muscle and the posterior abdominal wall. The cavity contains about an ounce of blood-tinged purulent fluid which infiltrates the muscle; no connection with the vertebrae or cord can be demonstrated.

Pleural cavities contain bloody fluid and present posteriorly some dense adhesions. The visceral pleura shows an adherent stringy fibrinous exudate. Right lower lobe is distinctly nodular and the cut surface drips a bloody fluid which in the region of the nodules is quite free from air bubbles. Cut surface of the nodules is a deep red, finely granular and projects slightly. The other lobes show an intense grade of edema but no naked eye evidence of consolidation.

The pericardial cavity contains about 3 ounces of clear yellow fluid. Heart is enlarged, particularly the right side, and the apex is somewhat blunted. Epicardium shows a marked excess of fat and a large number of pin-point to pea-sized petechiae. Myocardium is hypertrophied and the cut surface shows a number of grayish white to yellow flecks. Right chambers are dilated and contain fluid and clotted blood. Valve orifices are relatively dilated and the endocardium is free from vegetations.

Liver is somewhat reduced in size and presents a smooth glistening capsule through which the parenchyma appears as a distinctly yellowish substance. The consistence is increased and the cut surface shows a definite increase in interlobular connective tissue. A distinctly greasy fluid can be scraped from it.

The spleen is increased one and one-half the normal size. The capsule is tense and studded with innumerable pin-head to pea-sized hemorrhages. The substance is firm and the cut surface presents an extremely friable and readily disintegrated pulp in which the malpighian follicles are quite conspicuous from swelling.

The pancreas is normal in size and shape. The consistence is greatly increased, the substance cuts with resistance and the cut surface presents a diffuse, grayish, finely granular or even smooth tissue in which the normal lobulation is not apparent to the naked eye.

Adrenals appear to be somewhat enlarged and softened. The section reveals a greatly widened and blood infiltrated medulla.

The kidneys, bladder, testicles and gastrointestinal tract show no significant naked eye changes.

Removal of the calvarium reveals a most marked grade of subpial edema, the pia in places bulging from underlying accumulations of yellowish fluid. In other places there are seen through the membranes collections of greenish pus which is rather thick, fibrinous and adherent. The purulent meningeal infiltration is almost exclusively confined to the cerebral vertex. A large quantity of thin purulent fluid is present at the base. The vessels remain patent and the walls are atheromatous. Section of the brain reveals innumerable irregularly scattered punctate hemorrhages. Ventricles are dilated and distended with a milky fluid.

Cultures from the heart blood, from the retroperitoneal abscess and from the liver, spleen and brain reveal an organism morphologically and culturally identical with that isolated from the blood and spinal fluid during life and corresponding to the bacillus acidi lactici.

SUMMARY

A case of bacillus acidi lactici meningitis and septicemia is reported with isolation of the organism from the blood and spinal fluid before death and from the viscera and brain after death. There is no record in the literature of another case of bacillus acidi lactici meningitis.

The patient presented all the classical signs of meningitis and the pathogenicity of the organism was demonstrated by its virulence for rabbits and guinea pigs.

Thanks are due Dr. G. R. Lacy of the Singer Memorial Research Laboratory for his kind interest in independently confirming the bacteriological findings.

NOTE: Dr. Ray has prepared a full reference to the literature of this subject which cannot be published on account of its length.—*Editor.*

LABORATORY METHODS

THE RELATIVE VALUE OF SOME OF THE COMMONLY USED METHODS FOR THE DETECTION OF OCCULT BLOOD IN THE STOOL*

By Hobart A. Reimann, M.D., Buffalo, N. Y.

FROM the conflicting reports of various authors on the results obtained in testing for occult blood by some of the more commonly used methods at our disposal, it appeared advisable to test the relative value of these methods on a series of normal and pathological cases, the results of which are submitted below.

EXPERIMENT

Patients were selected from the wards, who had no evident lesion in the gastrointestinal tract and special care was taken to exclude pyorrhea, bleeding gums or hemorrhoids. Stools were tested for the presence of occult blood and the patients placed on a meat-free, chlorophyll-free diet, regardless of result, for the remainder of the experiment. After three or four days, all stools gave negative reactions.

Approximately 30 c.c. of blood was withdrawn from the median basilic vein, oxalated and placed in the ice box.

Each patient was then given an increasing dose of his own blood every day The stools examined, were marked with green corn or carmine in order to tell approximately whether the quantity of blood given the day before was in the sample of feces tested. Agar was occasionally used to insure a daily stool. Only formed stools were used.

The blood was given at the noon meal usually in a small quantity of cocoa or cocoa meringue. The total quantity of the mixture was small so that all would be eaten. When the doses of blood were greater than 3 c.c., one-half the dose was given at dinner and the other half at supper.

Technic.—The whole stool was thoroughly stirred and one gram placed in a test tube and stirred with 5 c.c. of water and 2 c.c. glacial acetic acid. Then 2½ c.c. of ether was added and the mixture was thoroughly shaken. Finally 2½ c.c. more of ether was added and the tube inverted 10-15 times slowly.

Benzidine test.—Equal parts (5 c.c.) of saturated solution of benzidine (10 per cent) and hydrogen peroxide were mixed and overlaid with the ethereal extract.

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TABLE I

RESULTS

CASE AND AMT'S OF BLOOD	BENZIDINE 10%	GUAIAC 1-60	BENZIDINE GREGERSON TECH.	GUAIACONIC ACID	SPECTROSCOPIC
J. S. Neurasthenia					
0 c.c.	neg.	neg.			
1 c.c.	very faintly pos.	neg.			
2 c.c.	very faintly pos.	neg.			
4 c.c.	strongly pos.	pos.			
Repeat					
0 c.c.	neg.	neg.			
4 c.c.	pos.	faintly pos.			
H. A. R.					
0 c.c.	very faintly pos.	neg.			
1 c.c.	neg.	neg.			
1½ c.c.	faintly pos.	neg.			
2 c.c.	strongly pos.	very faintly pos.			
2½ c.c.	strongly pos.	faintly pos.			
Repeat					
0 c.c.	neg.	neg.	neg.	neg.	
3 c.c.	pos.	neg.	pos. in 3 minutes	neg.	
4 c.c.	strongly pos.	faintly pos.	strongly pos.	neg.	
5 c.c.	strongly pos.	faintly pos.	pos.	very faintly pos.	
9 c.c.	strongly pos.	pos.	strongly pos.	very faintly pos.	
T. R. Nephritis					
0 c.c.	very faintly pos.	neg.			
1 c.c.	faintly pos.	neg.			
2 c.c.	pos.	neg.			
3 c.c.	faintly pos.	neg.			
4 c.c.	faintly pos.	neg.			
A. W. Br. Asthma					
0 c.c.	neg.	neg.	neg.	neg.	
1 c.c.	pos.	neg.	neg.	neg.	
1½ c.c.	neg.	neg.	neg.	neg.	
2 c.c.	strongly pos.	neg.	faintly pos.	neg.	
3 c.c.	neg.	neg.	neg.	neg.	
5 c.c.	strongly pos.	neg.	pos.	neg.	
6 c.c.	pos.	neg.	pos.	neg.	
F. K. Arterio-sclerosis					
0 c.c.	neg.	neg.	neg.	neg.	
1 c.c.	neg.	neg.	neg.	neg.	
1½ c.c.	pos.	neg.	faintly pos.	neg.	
2 c.c.	pos.	neg.	pos.	neg.	
3 c.c.	strongly pos.	neg.	pos.	neg.	
4 c.c.	strongly pos.	very faintly pos.	strongly pos.	neg.	
J. Y. Multiple neuritis					
0 c.c.	neg.	neg.	neg.	neg.	
2 c.c.		neg.	pos.	neg.	
3 c.c.		pos.	pos.	faintly pos.	
6 c.c.		strongly pos.	strongly pos.	very faintly pos.	

TABLE I—CONT'D.

RESULTS

CASE AND AMT'S OF BLOOD	BENZIDINE 10%	GUAIAC 1-60	BENZIDINE GREGERSON TECH.	GUAIACONIC ACID	SPECTROSCOPIC
F. B. Sciatica					
0 c.c.	neg.	neg.	neg.	neg.	
2 c.c.		neg.	faintly pos.	neg.	
3 c.c.		faintly pos.	pos.	neg.	
4 c.c.		neg.	faintly pos.	neg.	
8 c.c.		pos.	strongly pos.	very faintly pos.	
PATHOLOGICAL CASES					
C. G. Carcinoma stomach		pos.	strongly pos.	pos.	
J. K. Cholemic hemorrhage		very strongly positive	very strongly positive	pos.	Neutral methemoglobin bands
S. S. Carcinoma stomach		very strongly positive	very strongly positive	pos.	Faint neutral methemoglobin bands
N. A. Duodenal ulcer		pos.	strongly pos.	pos.	Faint acid hematin bands
J. S. Carcinoma stomach		pos.	strongly pos.	pos.	Faint acid hematin bands

The following are some quantitative results obtained with the various reactions when blood was added to originally negative stools:

PROPORTION OF BLOOD TO STOOL	GUAIAC	BENZIDINE GREGERSON TECH.	GUAIACONIC ACID	SPECTROSCOPIC
1-60	strongly pos.	strongly pos.	pos.	Acid haematin bands
1-84	strongly pos.	strongly pos.	pos.	neg.
1-120	pos.	strongly pos.	pos.	neg.
1-225	pos.	strongly pos.	faintly pos.	neg.
1-600	pos.	pos. in 1 minute	doubtful	
1-1200	faintly pos.	pos. in 3 minutes	doubtful	
1-2250	neg.	pos. in 4 minutes	neg.	
1-6000	neg.	pos. in 6 minutes	neg.	
1-12000	neg.	pos. in 8 minutes	neg.	

Guaiac test.—Gum guaiac, .08 gm., was dissolved in 5 c.c. alcohol (1-60 sol.) equal parts (5 c.c.) of this solution and hydrogen peroxide were overlaid with the ethereal extract.

Gregerson's test.—Benzidine, .02 gm., and .1 gm. barium peroxide were dissolved in 5 c.c. of 50 per cent glacial acetic acid and the solution overlaid with the ethereal extract.

Guaiaconic acid.—Two c.c. of the ethereal extract were treated with a solution of the preparation of guaiac, prepared according to the method of Lyle and Curtman,[1] and one to five drops of 30 per cent perhydrol was added drop by drop.

Spectroscopic.—The ethereal extract was examined by a direct vision spectroscope.

DISCUSSION OF RESULTS

With patients on a full unrestricted diet, practically every stool was positive to both benzidine and guaiac. The writer also included himself in the experiments and after a heavy meat diet gradually placed himself on a meat-free, chlorophyll-free diet. The stool from the heavy meat diet produced a positive benzidine and a faintly positive guaiac reaction. The stools became less and less positive to benzidine and negative to guaiac until the fourth day after the last meat was eaten, when both benzidine and guaiac reactions were negative. The result corresponded exactly to that of Dewis' experiment.[2]

The practice of introducing a patient's own blood into his stomach should correspond more or less to the behavior of blood from a bleeding ulcer or cancer, and would seem to be of much greater use in estimating the value of a test for occult blood than is the addition of blood to a stool after the latter has passed through the alimentary tract, and has gone through all the processes of digestion.

Theoretically, a patient receiving 1 c.c. of blood one day passes a stool containing the remains of 1 c.c. of blood the day following, and if 2 c.c. is given that day, he will pass the remains of 2 c.c. the next day and so on. But there is no way of telling how much of the stool has passed through or how much is retained from the day before, so that the results can never be exact. Furthermore the rate of digestion, the action of individual enzymes, the rate of bowel movement and the nature of the food eaten certainly all have a modifying effect on different individuals or in the same individual at different times. However, in the case of a marked stool in a normal individual with a normal evacuation, one may be reasonably certain of obtaining most of the hemoglobin ingested or rather the substance to which the reaction is due. It is evident that the stool must be thoroughly mixed to obtain a representative sample.

When preparing the ethereal extract we have found that a clear ether is always obtained if part of the ether is thoroughly shaken with the stool emulsion and the remainder is merely mixed by inverting the test tube ten to fifteen times. We found, in accordance with the finding of Dewis, that it is unnecessary to boil the mixture before extracting. Removal of fat is also unnecessary.

The original benzidine test, employing a saturated solution of benzidine gives a dark-colored underlying solution, so that faint reactions of green color are easily missed. We found the guaiac test to be on the whole, quite satisfactory and reliable and it is a valuable check to the benzidine reaction.

The technic proposed by Gregerson is the most reliable of the methods used and gives the most clean-cut reaction.

The use of guaiaconic acid prepared according to the formula of Lyle and Curtman[1] gave us positive results only with amounts of blood over 3 c.c. or in a dilution of 1-200 as shown in the control table, besides being

quite troublesome to make, but has remained constant in strength (in solution) for ten months after its first trial. In order to overcome the usual objection to guaiac, namely the lack of uniformity of gums, we purchased a considerable amount of powdered gum and thoroughly mixed it. This if used in exact quantities is reliable after knowing the strength of reaction to known amounts of blood.

The spectroscope in our hands was only of value when the blood in the stool was present in proportions of 1-60 and in confirming the presence of blood in large quantities, in which case it is the most definite of all tests.

It was found in accordance with most authors[4, 1] that the factors of paramount importance in accurate tests are the use of constant reagents and proportions and clean glassware. However it is only necessary to clean the test tubes used with scouring soap and hot water. The precise laborious chemical methods of Gattner and Schlesinger[5] were found superfluous. Another factor which must of course not be neglected in interpreting the test is as emphasized by many investigators, namely, the absolute necessity of keeping a patient on a meat- and chlorophyll-free diet for at least three days previous to the test.[3, 6, 8, 9]

As regards the delicacy of the reaction, we could not, as Cowie states, detect an intestinal hemorrhage of 1 c.c. by the guaiac method. We found that at least 2 c.c. is necessary.

As to the spectroscopic examination our findings also differ materially from those of Boas.[10] According to the latter, the spectroscopic findings are identical with that of the Gregerson technic and 2 c.c. of blood introduced into the stomach can be detected. We have obtained positive benzidine reactions (Gregerson technic) with 2 c.c. of blood also but we found that over 6 c.c. are necessary before the hemochromogen stripe can be obtained in the spectroscope. Our principal difficulty was the density of the color of the ethereal extract which tended to obscure the absorption bands, and removal of the color without removal of the hematin is not possible. Von Domarus[14] states that the hemoglobin derivative bands are best seen when the dilution of the substance lies between 1-50 and 1-200.

Grünwald finds that the benzidine test is positive with 1 part of blood in 150,000, guaiac with 1 to 2000 and the spectroscope 1 in 5000.

Our results show the benzidine test (Gregerson technic) positive with 1 part of blood to 12,000, guaiac with 1 in 1200, spectroscope 1 part to 60.

Lyle and Curtman[1] find that their preparation is positive to a 1 to 10,000 solution of blood. In our hands it becomes definitely positive only when the dilution of blood is 1-225, greater dilutions being doubtful, on account of the color of the ethereal extract, or entirely negative.

It seemed that the best and most convenient method to date is the Gregerson technic, checked by the 1 to 60 solution of guaiac. For convenience we have powder papers made containing .02 gm. benzidine and .1 gm. barium peroxide and powder papers containing .08 gm. of powdered gum guaiac. When dissolved in 50 per cent glacial acetic acid and 95 per cent alcohol respectively, these make solutions of proper and constant strength, and are al-

ways ready to use. We agree with Wolf[6] that benzidine (Gregerson technic) is a test delicate enough but not too delicate and guaiac a test not too coarse to ascertain the usual amounts of occult blood found in the stool.

It seems perfectly possible that, with the use of constant chemicals giving reactions to quantities of blood known beforehand, the quantity of blood passed in feces could be approximately determined. Thus the carefully controlled benzidine and guaiac reactions could be made quantitative tests, making it possible to estimate the rate of hemorrhage. Other investigators have recently brought forth the same view.[12]

If the objection of the hypersensitiveness of the benzidine test is eliminated by the routine control with the guaiac test we possess in it a very reliable test for occult blood. Certainly more useful than the spectroscopic test so strongly recommended by Snapper[13] and others.[10]

CONCLUSIONS

1. A positive reaction for occult blood in the stool with any of the methods in use is, in accordance with most authors, valueless, when the patient has not been put on a meat- and chlorophyll-free diet and has not had thorough evacuations for at least three days prior to the test.

2. The most reliable and convenient test for occult blood in the stool is the Gregerson benzidine technic.[6, 10] The use of the guaiac test in conjunction with it is recommended in order to gain some idea of the quantity of blood if the amount present be between 1 and 5 c.c.

3. The spectroscope is not recommended for the detection of traces of blood.

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THE QUANTITATIVE ESTIMATION OF IODINE IN URINE*

By H. LaRue Marsh, M.S., Chicago, Ill.

IT FREQUENTLY becomes desirable to determine iodine eliminated during iodine therapy in a twenty-four-hour specimen of urine. None of the methods described in the literature are wholly satisfactory. Since some of the iodine is eliminated in organic combination, it is obviously necessary to precede an analysis with a fusion of an evaporated sample of urine.

The method described below is recommended not only as one embodying the very necessary fusion, but also as one well adapted to the usual apparatus, reagents, and experience of the clinical chemist. It consists of: first, the determination of the total halogens in the residue from a fused sample of urine by the Volhard-Arnold[1] volumetric method; second, the determination of the chlorides (Volhard-Arnold) in another fused sample from which the iodine has been removed by the classical method of Gooch.[2] The difference between the total halogens and the chlorides represents the iodine present in the sample of urine.

PROCEDURE

Fifty c.c. samples of the urine are evaporated to dryness in nickel or silver dishes with 35 c.c. of a solution of a fusion mixture containing 40 gm. of sodium hydroxide and 200 gm. of sodium nitrate per liter. This evaporation can be best done in a gas oven. When dry, the residues are fused till free from carbon, cooled, and dissolved in not more than 100 c.c. of distilled water. During fusion the dishes should be covered with watch glasses to avoid possible losses by spattering.

The total halogens are determined in one sample. The solution is quantitatively transferred to a 250 c.c. volumetric flask and 110 c.c. of tenth normal silver nitrate are added from a burette, after which 15 c.c. of concentrated chloride-free nitric acid are added. The addition of the acid *after* the silver nitrate avoids possible losses of iodine. The solution is now shaken till effervescence has ceased, made up to the mark, mixed and filtered through a dry filter into a dry container. One hundred c.c. of the clear filtrate are transferred to a 250 c.c. Erlenmeyer flask, 1 c.c. of a saturated solution of chloride-free ferric alum is added, and the excess of silver nitrate present is titrated against a tenth normal potassium sulphocyanate solution till the first permanent coloration is produced.

The chlorides are determined in a second sample, the fusion of which having been dissolved, is transferred to a two-liter Erlenmeyer flask and

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diluted to a volume of approximately one liter with distilled water. To this is added 3 c.c. of concentrated chloride-free sulphuric acid and 10 c.c. of dilute nitrous acid, chloride free (or 0.5 to 1.0 gram of chloride-free sodium nitrite). The solution is boiled until all the iodine has been expelled and the solution has become colorless. In all cases the solution should be boiled for at least thirty minutes. One hundred ten c.c. of tenth normal silver nitrate are now added from a burette. The addition of the silver at this point is necessary to prevent loss of chlorides during subsequent concentration. The solution is boiled down to a volume of 200 c.c. or less. (Bumping may be avoided and evaporation hastened by passing a current of air through the solution.) When concentrated sufficiently, the solution is cooled to room temperature and transferred to a 250 c.c. volumetric flask and made up to the mark with distilled water. From this point the procedure is exactly the same as described for the first sample.

The iodides present are calculated from the differences between the total halogens and the chlorides. The method used on samples of urine containing known quantities of sodium iodide gave results (average of duplicate determinations) as shown in Table I:

TABLE I

SAMPLE OF URINE NUMBER	GRAMS OF NaI PER 100 C.C. OF URINE PRESENT	GRAMS OF NaI PER 100 C.C. OF URINE FOUND
1	0.1098	0.1089
2	0.1098	0.1086
3	0.1098	0.1089

In conclusion it may be stated that the above described method is simple in technic and gives dependable and accurate results.

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A SIMPLE METHOD FOR CALCULATING THE BASAL METABOLIC RATE*

BY RUSSELL L. HADEN, M.A., M.D., KANSAS CITY, KANSAS

BY common consent the results of the basal metabolism determination are expressed in terms of calories of heat produced per square meter of surface area per hour. It is usually assumed that the respiratory quotient is 0.82, hence 4.825 is the number of calories taken as resulting from the consumption of a liter of oxygen. In the closed circuit type of apparatus such as the Benedict the metabolic rate is determined from the amount of oxygen consumed over a certain period of time. For many workers the calculation

*From the University of Kansas, School of Medicine, Kansas City, Kansas.
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TABLE I

The factor at any temperature and pressure represents the following: $\frac{TC \times BC \times 60 \times 4.825}{1000}$ where TC is the temperature correction and BC is the barometer correction.

P. (m.m.)	15°	16°	17°	18°	19°	20°	21°	22°	23°	24°	25°	26°	27°	28°	29°	30°
700	0.249	0.248	0.247	0.246	0.245	0.244	0.242	0.241	0.240	0.239	0.238	0.236	0.235	0.234	0.233	0.232
705	0.251	0.250	0.248	0.247	0.246	0.245	0.244	0.243	0.242	0.240	0.239	0.238	0.236	0.235	0.234	0.233
710	0.253	0.252	0.250	0.249	0.248	0.247	0.246	0.245	0.244	0.242	0.241	0.240	0.238	0.237	0.236	0.235
715	0.254	0.253	0.252	0.251	0.250	0.249	0.247	0.246	0.245	0.244	0.243	0.241	0.240	0.239	0.238	0.237
720	0.256	0.255	0.254	0.253	0.252	0.251	0.249	0.248	0.247	0.246	0.245	0.243	0.242	0.241	0.240	0.239
725	0.258	0.257	0.256	0.255	0.254	0.253	0.251	0.250	0.249	0.247	0.246	0.245	0.244	0.243	0.241	0.240
730	0.260	0.259	0.257	0.256	0.255	0.254	0.253	0.251	0.250	0.249	0.248	0.246	0.245	0.244	0.243	0.242
735	0.262	0.261	0.259	0.258	0.257	0.256	0.255	0.254	0.252	0.251	0.250	0.248	0.247	0.246	0.245	0.244
740	0.264	0.263	0.261	0.260	0.259	0.258	0.256	0.255	0.254	0.253	0.252	0.250	0.249	0.248	0.247	0.246
745	0.265	0.264	0.263	0.262	0.261	0.259	0.257	0.256	0.255	0.254	0.253	0.251	0.250	0.249	0.248	0.247
750	0.267	0.266	0.265	0.263	0.261	0.259	0.257	0.256	0.255	0.254	0.253	0.251	0.250	0.249	0.248	0.247
755	0.268	0.267	0.266	0.265	0.264	0.263	0.261	0.260	0.259	0.258	0.257	0.255	0.254	0.253	0.251	0.250
760	0.270	0.269	0.268	0.267	0.266	0.264	0.263	0.262	0.261	0.259	0.258	0.257	0.255	0.254	0.253	0.252
765	0.272	0.271	0.270	0.268	0.267	0.266	0.265	0.264	0.263	0.261	0.260	0.259	0.257	0.256	0.255	0.254
770	0.274	0.273	0.272	0.270	0.269	0.268	0.267	0.265	0.264	0.263	0.262	0.260	0.259	0.258	0.257	0.256
775	0.276	0.275	0.273	0.272	0.271	0.270	0.268	0.267	0.266	0.265	0.264	0.262	0.261	0.259	0.258	0.257
780	0.278	0.277	0.275	0.274	0.273	0.272	0.270	0.269	0.268	0.267	0.265	0.264	0.262	0.261	0.260	0.259

from the data thus obtained is more laborious and time consuming than the actual running of the test.

The calculation requires first of all the reduction of the oxygen as measured to 0° C. and 760 mm. barometric pressure. This corrected gas volume is then multiplied by 60 to give the oxygen consumption per hour and next by the factor to obtain the total calories of heat produced. The resulting figure is finally divided by the surface area as determined from the height and weight by the formula or graph of DuBois[1] (Fig. 1). This final result is the basal metabolic rate. It has only to be compared with the DuBois standards for sex and age[2] (Table II) to determine whether it is plus, minus or normal.

McCaskey[3] gives a table for making the temperature and pressure cor-

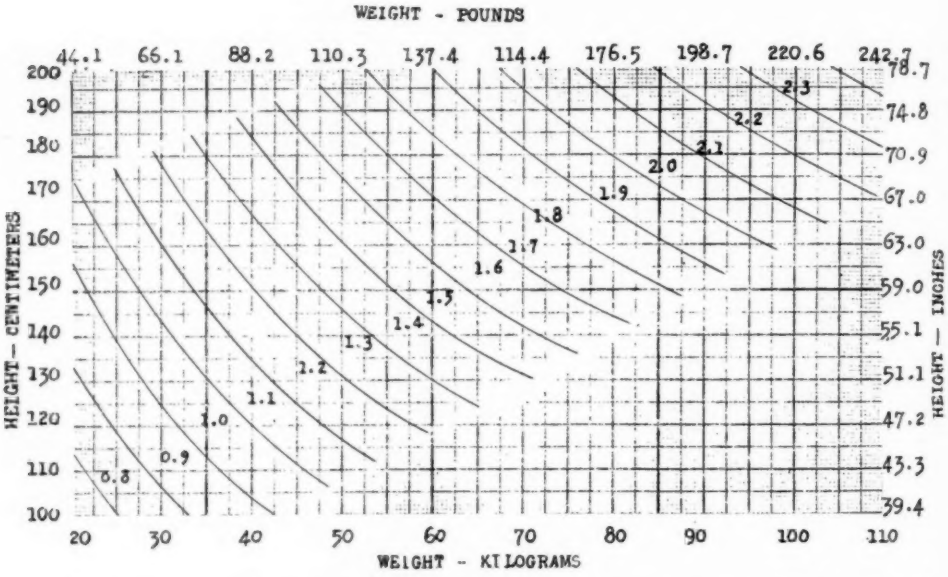

Fig. 1.—Chart for determining surface area of man in square meters from the weight in kilograms (Wt.) and height in centimeters (Ht.) according to the formula: Area (Sq. Cm.) = $Wt.^{0.425} \times Ht.^{0.725} \times 71.84$. (DuBois). The equivalents of the centimeters in inches and the kilograms in pounds are given.

rections. The table is correct only when soda lime which will absorb moisture is employed in the test. Newcomer[4] has suggested several aids in calculation based on logarithmic tables. These for the ordinary person are confusing rather than helpful. Similarly Smith[5] has worked out a graph for calculating the metabolic rate in terms of cubic centimeters per minute instead of calories per hour.

For the past two years I have used a table which simplifies the calculation of the basal metabolic rate a great deal. Formerly the table was based on the assumption that the air in the apparatus is practically moisture free, and vapor tension can be ignored. This is true only when caking soda lime or an additional bottle of calcium chloride is employed. At the present time almost every one is using the noncaking soda lime as perfected by Wilson.

TABLE II

STANDARDS OF METABOLISM (DU BOIS)

AGE	MALE (Calories per hour per sq. m.)	FEMALE (Calories per hour per sq. m.)
14-16	46.0	43.0
16-18	43.0	40.0
18-20	41.0	38.0
20-30	39.5	37.0
30-40	39.5	36.5
40-50	38.5	36.0
50-60	37.5	35.0
60-70	36.5	34.0
70-80	35.5	33.0

This absorbs very little moisture so the air in the apparatus is about 80 per cent saturated. I have recalculated my table (Table I) on the basis of the table given by Wilson[6] for the temperature and pressure correction of 80 per cent saturated air.

In making the calculation the total amount of oxygen consumed during the period of the test is divided by the number of minutes the test is run to give the oxygen consumption per minute. The factor corresponding to the temperature and nearest barometer reading is found in Table I. This factor represents the following:

$$\frac{TC \times BC \times 60 \times 4.825}{1000}$$

where TC is the correction for temperature and BC is the correction for barometric pressure.

The product of this factor by the oxygen consumed per minute in cubic centimeters represents the total heat production. This has only to be divided by the surface area as determined from the DuBois chart and compared with the normal for that age and sex.

The calculation is best illustrated by an example. The patient is a woman age 17. In a ten-minute period 2610 c.c. of oxygen are consumed, or 261 c.c. per minute. The temperature is 27° C. and the pressure is 745 mm. The factor corresponding in the table is 0.250. The total heat production is: $261 \times 0.250 = 65.2$ calories. The weight of the patient is 132 pounds, and the height 5 feet 5 inches, so the surface area is 1.63 square meters. The basal metabolic rate is: $\frac{65.2}{1.63} = 40$ calories per square meter per hour. This is just normal for a girl of 17 as will be seen from Table II.

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AN APPARATUS FOR CLEANING BLOOD COUNTING PIPETTES*

By Russell L. Haden, M.A., M.D., Kansas City, Kansas

FOR accurate blood counting it is absolutely necessary that the pipettes used be carefully cleaned and dried. The process is a tiresome and time-consuming one if many pipettes are to be cleaned. Most laboratory workers make use of some type of suction apparatus usually employing a piece of rubber tubing attached to a water suction pump. Gray† describes such a method by which one or more pipettes may be cleaned and dried. He sug-

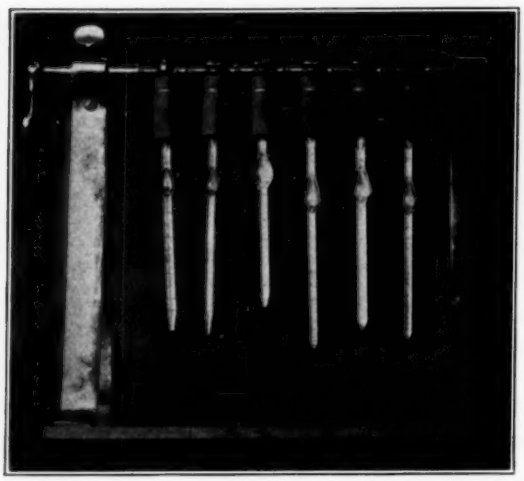

Fig. 1.

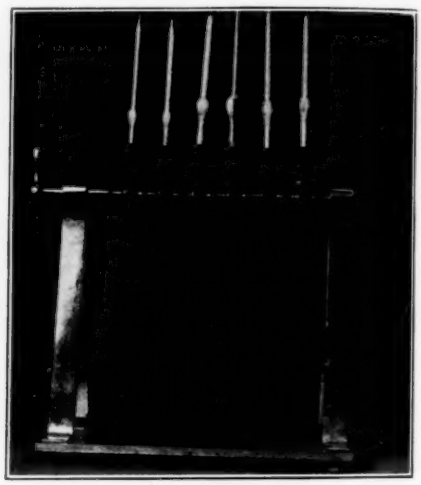

Fig. 2.

Fig. 1.—The apparatus is shown in position for filling the pipettes with water, alcohol or ether. The rubber tube at the right connects with the suction pump.
Fig. 2.—Apparatus in position for emptying pipettes.

gests that the pipettes be dried by gently heating while suction is applied.

I have for some time been using an apparatus with which one or more pipettes can be cleaned and dried thoroughly and rapidly. The device is illustrated in Fig. 1. It is made by soldering the desired number of three-way stopcocks together. Six is enough as the cleaning can be done so quickly more are seldom necessary. The best type of stopcock is the small one made to fit the tip of a Luer syringe. The stopcock must be so made that the air can be drawn straight through and in by the side inlet at the same time.

One end of the soldered stopcocks is closed with a plug or with solder, the other is connected by a piece of rubber tubing to a water suction pump. The apparatus should be supported on a metal frame set on a heavy metal

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†Gray, H., Jour. Am. Med. Assn., 1921, lxxxvi, 1826.

base. A handle is attached at the closed end so the pipettes can be tipped up for emptying. To each side inlet tube a short piece of pressure tubing is attached in the lumen of which the pipette is placed.

After all the pipettes to be cleaned have been placed in position the suction is turned on and water is run through each pipette. The handle is then turned so that the pipettes are vertical with the tips in the air (Fig. 2). The pipettes may be dried by flaming gently with Bunsen burner as suggested by Gray or by running through in succession alcohol and ether.

FORMALDEHYDE TEST IN LUES*

By D. A. Johnston, M.D., Cincinnati, Ohio

IN the search for a chemical test for lues, Gate and Papacosta of Italy noticed that addition of liquor formaldehyde to blood serum caused it to gel in the presence of syphilis, and otherwise not.

Blood is drawn as for a Wassermann. At the end of twenty-four hours the serum is decanted from the test tube into another clean but not sterilized tube. A drop of ordinary commercial liquor formaldehyde is added, and the tube plugged with cotton. The serum and formaldehyde are allowed to remain at ordinary room temperature for twenty-four hours. At the end of this period observation is made as to the condition of the serum. Coagulated serum is a positive result; fluid serum is negative.

Echer of Cleveland reports 37.09 per cent of plus formal tests agreed with plus of Wassermann, 44 or 8.8 per cent of formal plus were of plus type, and of these 13, or 29.54 per cent were Wassermann plus.

Kingsbury, London, says that less than one-half of serums giving plus Wassermann, show coagulation with formalin and nearly 10 per cent of nonsyphilitic serums give a plus result.

A. Terzani, Florence, Italy, obtained conflicting findings in 226 tests with this method.

A. Bouttian, Paris, concludes from 1500 applications of the test that the reaction is strictly specific for syphilis.

My series of 100 cases is composed of 100 consecutive male admissions to the hospital, all being insane patients. The results are not only interesting for what they disclose as to the value of the formaldehyde test but also for what they show relative to the incidence of lues in general among the insane commitments.

In our Wassermann tests we employ the ice box fixation method, as well as the water-bath, using both the plain and cholesterinized antigen.

In 27 per cent the Wassermann was plus; in 23 per cent the formaldehyde test was plus; in 12 per cent the two tests correspond; in 11 per cent the for-

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maldehyde was plus with negative Wassermann findings; in 15 per cent Wassermann was positive with negative formaldehyde test.

From this will be seen the tendency to induce confusion, and the fact that the test is of no diagnostic value because of the failure to react clinically and serologically in clear cut cases of lues, and also because of the occurrence of plus reactions in absence of disease.

The following table summarizes the psychotic findings:

PSYCHOSES REPRESENTED IN THE 100 CASES

Paresis	26	Arteriopathic	3
Constitutional Psychopathic Inferior	14	Alcoholic	3
Dementia Praecox	12	Involution Melancholia	2
Senile Dementia	10	Psychoses with somatic diseases	2
Manic Depressive	9	Narcotic	2
Paranoic Conditions	6	Epileptic	1
Psychoses with mental deficiency	6	Huntington's Chorea	1
Psychoneuroses	3		
		Total	100

A SUGGESTION FOR A PRACTICAL SPIROMETER VALVE*

By G. A. Brough, A.B., B.S., Chicago, Ill.

TO accurately determine tidal air with the spirometer an efficient valve is necessary. Any valve which requires considerable energy for operation —which presents obstruction—increases the normal breathing movements. Likewise a valve which permits escape of air during slight movements or does not close promptly is very unsatisfactory. To eliminate some of the unsatisfactory features of the flap valve (Chauveau), and the liquid valve (Müller), a membrane valve was constructed.

The construction can be seen from the accompanying diagram which is almost self-explanatory. The only adjustments necessary are the contacts of the glass outlet (a) with rubber dam (b), and at the inlet (c) and (d). The rubber dam covers the end of a glass cylinder held in place by three pieces of rubber tubing. The glass outlets and inlets are held by rubber stoppers which have been cut to fit.

The valve is reliable, always opening and closing, and is entirely free from the disadvantage of sticking open or closed; the air resistance to the breathing movements has been reduced to a minimum; the valve operates during the most feeble respiratory movements and does not leak, however slight such movements may be; the operation is instantaneous with the initiation of any movement of air; no operative adjustments necessary and no

*From the Laboratory of Pharmacology and Therapeutics, University of Illinois, College of Medicine, Chicago, Illinois.

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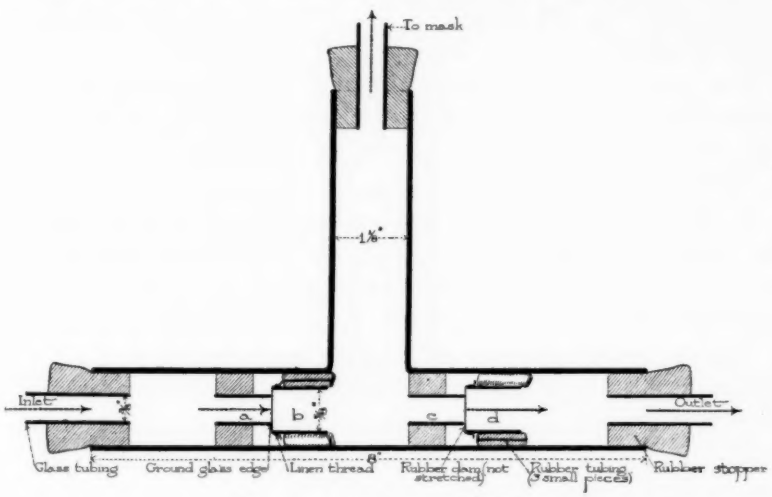
moving mechanical parts to wear after valve is assembled; moisture was found to be beneficial to the action; and from the standpoint of convenience in ex-



Showing construction of Valve.

Fig. 1.

perimentation it may be noted that the valve will operate in any position.

The valve units may be assembled in short tubes and connected by means of a T-tube. This permits ready change of direction and rapid replacement.

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CONCERNING THE SPECIFICITY OF CHOLESTERINIZED ANTIGENS IN THE SEROLOGIC DIAGNOSIS OF SYPHILIS: THIRD COMMUNICATION*

By Robert A. Kilduffe, A.M., M.D., Pittsburgh, Pa.

IN previous communications concerned with this subject,[1, 2] the following facts were emphasized:

1. That cholesterinized antigens seemed destined to form a permanent part of the complement-fixation technics devised for the serologic diagnosis of syphilis.

2. That, if such antigens were prone to give nonspecific and false fixations in a large proportion of cases, the factor of error thus introduced forbade their use as the sole antigen in any technic utilized for the serologic diagnosis of syphilis.

3. That complement-fixation technics relying entirely upon cholesterinized

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antigens for the detection of syphilitic reagin were, nevertheless, rather widely used.

4. That, therefore, the evolution of a concerted opinion as to their reliability was indicated.

The only means whereby such an opinion can be materialized seems to be by the collection and analysis of data concerned with the establishment of the reliability or nonreliability of cholesterin-plus reactions as a serologic indication of syphilis.

In the previous articles an attempt was made in a small way to gather data concerned with this problem, and the purpose of the present communication is to present further data of like character.

As a result of an investigation into the merits or demerits of a new complement-fixation technic recently proposed for standard adoption by Kolmer[3], the following conclusions, among others, were deemed warranted:

1. That the Kolmer modification possessed great delicacy.
2. That insofar as the series showed, false positives did not occur.
3. That while, because of factors inherent in and concerned with the formation or production of syphilitic reagin, the method was not infallible, occasionally failing to detect its presence, the occurrence of a positive reaction by this technic constituted very strong presumptive evidence of the presence of syphilitic reagin almost invariably, when evidence was obtainable, corroborated by clinical and other findings.

Under these circumstances it seemed of interest to note the results obtained with cholesterin-plus serums when tested by what seems to be a most delicate and reliable technic.

It may be argued that as the antigen devised by Kolmer[4] is a polytropic cholesterinized extract to which has been added acetone-insoluble lipoids, the comparison of the two methods is of little value as affecting the status of cholesterinized antigens. The new antigen, however, from the standpoint of delicacy and specificity is so far in advance of any with which the writer is familiar as to be practically different and distinct from the ordinary plain alcoholic extract cholesterinized and the comparison of the results obtained by the two methods seems, therefore, justifiable.

In the course of this investigation 1014 serums were examined by two methods the details of which have been previously published.

There were in the series 91 serums reacting only to the cholesterinized extract (alcoholic extract of human heart with 0.4 per cent cholesterin). In 64 of these or 70 per cent a positive reaction was also obtained with the Kolmer modification. Of these 64 cases 43 were known to be syphilitic, many being cases under treatment; in 10 cases no data was obtainable; and 11 were prostitutes in whom the possibility of syphilis cannot be overlooked.

There remain 27 serums with which negative reactions were obtained by the Kolmer modification.

It has been noted above that the analysis of the results obtained with the Kolmer method lead to the conclusion that a positive reaction by this method

constitutes very strong presumptive evidence of the presence of syphilitic reagin.

The same degree of reliance, however, cannot be placed in a single negative reaction by this—or any other—technic as indicative of the absence of syphilitic reagin, so that it is possible that syphilitic reagin might be truly indicated by the cholesterin-plus reaction and the same serum still negative—*on a single examination*—by a more delicate and specific method.

This fact of the inconstant production and presence of syphilitic reagin; the fact that it is not produced in mathematical proportion in various stages of the disease, and that unaccountable and inexplicable variations in the reactions obtained occur, constitute one of the inherent factors of error in complement-fixation reactions upon an appreciation of which is based the agreement upon the necessity for more than one negative test before the absence of syphilitic reagin can be reasonably maintained.

The fact that a negative Kolmer reaction was obtained in these 27 serums is not sufficient evidence, therefore, upon which to assume their nonspecific reaction with the cholesterin antigen in the other technic.

Owing to circumstances beyond control no historical or clinical data for or against the presence of syphilis in these cases is at hand and the question must be left in doubt.

The high percentage of agreement in the other 64 cases, however, coupled with the fact that these serologic findings were corroborated in a large proportion of these cases, seems sufficient evidence, in conjunction with what has been previously presented, upon which to reiterate the claim that the cholesterin-plus reaction cannot be justifiably looked upon as without significance; and to again urge the collection and analysis of a sufficiently extensive series of such reactions to place the question upon a sound and agreed footing.

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EDITORIALS

Bismuth as a Substitute for Arsphenamine

THE use of bismuth in the treatment of syphilis was developed in France chiefly through the efforts of Sazerac and Levaditi, who recognized the value of the earlier uncompleted work of Sauton and Robert of the Pasteur Institute. The preliminary observations made by Sauton and Robert were published in Annales de L'Institut Pasteur in 1912. No further experimental work was performed until after the war when in 1921, Sazerac and Levaditi published their preliminary observations. In January 1922, a symposium on the use of bismuth in the treatment of syphilis appeared in the Annales de L'Institut Pasteur. Between January and October 1922, twenty-three articles on the use of bismuth in syphilis have been recorded in the quarterly cumulative index, coming from the following eight countries: Argentina, Denmark, Italy, Brazil, Uruguay, Germany and Switzerland. During the same period the treatment was not discussed in the American medical press.

Sauton and Robert observed that infection of hens with spirocheta gallinarum may be prevented by the preliminary intramuscular injection of a bis-

muth emetin compound. Treatment of the disease with bismuth compounds gave promising but not conclusive results. Sazerac and Levaditi carrying this work forward, experimented chiefly with sodium-potassium tartrobismuthate. They found that this compound could be administered to rabbits in a dosage of from 50 to 60 mg. per kgm. body weight without deleterious effect, when given subcutaneously or intramuscularly. A dose of 200 mg. resulted in death within two to four days. Intravenously the drug is highly toxic, 5 mgs. causing death at the end of a week. The drug was first administered in watery solution and later suspended in oil, with more satisfactory results. The lessened toxicity from intramuscular injection is apparently due to temporary fixation of bismuth in the tissues, with resulting delayed absorption. On dissection, an extensive white deposit is found in the connective and muscular tissue. Müller of Mainz has corroborated this finding and has shown in roentgenograms taken on successive days, that the bismuth casts a shadow of decreasing intensity for three days after intramuscular injection.

The toxicity of an oily suspension is less than that of an aqueous solution of tartrobismuthate administered in the same manner. Oral administration of the drug had no effect upon the progress of syphilis in the experimental animal. Rectal administration caused a temporary but not permanent disappearance of the syphilitic lesions. This method of treatment is being studied more thoroughly. Analogous results were obtained by inunctions. The authors point out the possible advantages to be gained in prophylaxis from the use of bismuth ointment.

Sazerac and Levaditi have utilized various other combinations of bismuth, such as the ammoniacal citrate, the soluble lactate, the subgallate and oxyiodogallate, but found each to be either less active or more toxic than the tartrobismuthate of sodium and potassium.

In the treatment of human syphilis these authors report very promising results, the drug is unusually stabile and of low toxicity when given subcutaneously or intramuscularly. Its intravenous administration is absolutely contraindicated. They recommend the use of a suspension in oil.

Fournier and Guénot have collaborated with Sazerac and Levaditi in the clinical use of sodium-potassium-tartrobismuthate. They give the drug intramuscularly in a 10 per cent suspension in olive oil and emphasize the importance of depositing it into the muscles rather than subcutaneously. The latter method is decidedly more painful while the inconvenience from intramuscular injection is no greater than that observed after intramuscular mercury. A total of two or three grams of bismuth should be given during the first month of treatment. Two or three injections of 0.2 grams are given daily, after which the patient receives 0.3 grams twice weekly throughout the month. After this period, treatment may be continued with weekly injections of 0.2 to 0.3 grams or the patient may be allowed one month rest, after which the regular course is repeated. The only necessary precaution for the patient to observe is a careful hygiene of the mouth.

Under this treatment spirochetes disappear from the primary sore after the first to the third injection. The chancre becomes completely healed within

five to twenty-five days, usually within two weeks. The Wassermann reaction, if negative at the initiation of treatment, usually remains so. If positive, the strength of the reaction falls as satisfactorily or perhaps more so than after the use of arsphenamine.

Bismuth is particularly useful in the contagious stages and according to the authors, causes more rapid and complete disappearance of the contagious lesions, than does arsphenamine. In secondary syphilis the cutaneous lesions disappear as a rule within a week. The strength of the Wassermann reaction is very favorably influenced, becoming practically negative within two or three months as a rule, but varying considerably as with other forms of treatment. Tertiary lesions improve with almost equal rapidity. This is particularly true of gummata and tertiary skin and mucous membrane manifestations. The authors report no conclusive observations on visceral or nervous syphilis.

No untoward effects from the use of bismuth have been reported other than a tendency toward stomatitis similar to but usually not as severe as that following the use of mercury. A marginal pigmentation usually appears in the gums, analogous to the familiar lead line. The stomatitis is usually a fuso-spirillary infection similar to that of Vincent's angina. It is satisfactorily treated by the methods customarily employed in Vincent's angina and may even be rapidly cured by the local application of the tartrobismuthate in powder form. Bismuth appears in the saliva but in an altered form, probably combined with sulphur, in which it has lost its spirillicidal properties.

The drug has been found present in the blood and the cerebrospinal fluid, and it is eliminated in the saliva, the bile, feces, sweat, milk and in the urine. It appears in the urine within from eighteen to twenty hours after injection and persists for from twenty to thirty days after discontinuance of the course of treatment.

Marie and Fourcade have used the drug in general paralysis without distinct improvement. The results are analogous to those following neoarsphenamine and mercury. The spinal fluid Wassermann reaction remains uninfluenced although the blood Wassermann is improved. The cases were well advanced when treatment was instituted.

Hugo Müller, director of the skin and venereal clinic at Mainz, has corroborated the work of the French observers and reports equally satisfactory results. He reports unusually good results in the treatment of secondary syphilis accompanied by hypertrophic papules and rupeal syphilis, which are ordinarily rather highly resistant to the usual methods of treatment. He points out, also, that the drug is particularly useful in those cases where the spirochete has become arsenic resistant. Such cases clear up rapidly under bismuth treatment. He reports for example one patient who developed severe mercury poisoning from 1 gram of gray ointment, intense iodism after taking 0.5 grams of potassium iodid, and an acute dermatitis lasting two months following a dose of 1 gram of neosalvarsan.

Grenet, Drouin and Richon have produced an aromatic bismuth compound of the probable formula $C_{12}\ H_7\ O_6\ Na_3\ (COOBi\ (OH)^2\ )^2$, which may be injected intravenously without toxic effect.

The immediate results from the injection of bismuth compounds in syphilis appear from the published reports to be as good as or better than those obtained with mercury and arsenic compounds. In any case, the drug will be valuable for alternative use with the latter, in those cases where the infecting parasite has apparently acquired a degree of tolerance to the drug. It will be several years before the end results from bismuth treatment can be comprehensively tabulated. In fact it has not been until recently that we have come to realize the significance of the late unsatisfactory accidents from standard treatment with mercury and arsphenamine.

The discovery of the treponema pallida by Schaudinn and Hoffmann, the cultivation of the spirocheta by Noguchi, the application of the Bordet-Gengou phenomenon to the serologic diagnosis of syphilis by Wassermann and the preparation of salvarsan by Ehrlich have concentrated our attention on attempts to rid the body of the spirochete and to obtain a serologic cure, and have diverted our thoughts from the patient as an individual, so that in many instances we have lost sight of the importance of clinical cure. This is often not obtained until long after the Wassermann reaction has become negative.

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—*W. T. V.*

A New Liver Function Test

IN an editorial discussion of liver function in the June number of this Journal, it was suggested that functional tests carried out on blood alone would eliminate some of the confusion arising from coincident renal or biliary tract abnormality. This method of procedure should theoretically give more accurate information concerning certain phases of liver function. Since that time Rosenthal has published three reports on a new method for testing liver function with phenoltetrachlorphthalein. In the first he describes experimental results, devoting particular attention to the rationale and procedure of the test; in the second he reports his experiments on animals; and in the third, he summarizes the clinical results observed in man.

Five mg. of phenoltetrachlorphthalein per kgm. body weight is injected intravenously and the rapidity of the disappearance of the dye from the blood serum is determined. Normally within fifteen minutes, but 2 to 6 per cent is recovered, practically all being in the serum and none absorbed into the red blood cells. After from forty to sixty minutes the dye is no longer present in the circulation. In hepatic derangements high percentages may be found in the plasma many hours after injection. The standard for comparison is easily prepared by adding stated amounts of the dye to the patient's serum obtained before injection of the test substance. Sera obtained at the end of fifteen-minute and longer intervals are compared with the standard.

Rosenthal finds the highest grades of retention in acute hepatitis, in atrophic cirrhosis and in advanced carcinoma of the liver. In jaundice the concentration of the dye was not found to parallel the intensity of the icterus. Normally none appears in the urine. In hepatic disease, from a trace to 4 per cent was usually present but the percentage gave no indication of the degree of retention in the blood, and in some cases showing blood retention no dye appeared in the urine.

Following widespread parenchymal damage from experimental chloroform or phosphorus poisoning, highest degrees of retention in the blood stream were observed. When repair had taken place curves approaching normal were again obtained.

Following ligation of the common duct the dye was removed from the blood stream within practically the normal interval except that a trace remained present throughout the experiment. Rosenthal observes that in jaundice due to mechanical obstruction, the curves differ widely from those found in jaundice associated with extensive degeneration of the hepatic cells.

Rosenthal has also developed a "ring test" which he finds more sensitive than gross mixing of serum and reagent.

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—*W. T. V.*

Position of Doctors in the Province of Nikolaev, Ukraine

THROUGH the courtesy of Dr. Haigh, a member of the Health Committee of the League of Nations and health expert attached to the Nansen Organization, we have the following information concerning the position of doctors in the above-mentioned province. He states that the assistance given to these doctors has so far been negligible and that no organization for their relief exists in the province. A few of these doctors have friends abroad and occasionally receive parcels of foodstuffs. Their salaries are paid after long delays. Their tragic position continues to grow worse. During the winter of 1921-1922 some supplies were officially distributed among them, but even this relief has now come to an end. Many doctors are able to subsist only by the sale of their furniture or other possessions. Even those who have the best practices are in need of clothing. Those who live in remote districts have to rely on the help of such peasants as have managed to escape ruin. Without the official ration, which consists of a pound of maize distributed among a portion of the staff in the hospitals, life would be impossible in these establishments. In September, 1922, the hospital doctor received a salary of twenty-seven million rubles, equivalent to about $5. Since that time the value of money has decreased. Nurses receive about twenty million rubles and other members of the staff still less. Many doctors and nurses have died at their posts.

—*V. C. V.*

The Present Endemicity of Yellow Fever

BEFORE his death General Gorgas dreamed that he might see and might participate in the complete eradication of yellow fever from the earth. This was a reasonable ambition, since he had accomplished so much in this direction. When Gorgas began his work in Cuba a large percentage of foreigners coming to that Island was attacked by this disease and many succumbed. At that time yellow fever was widely distributed through the West Indies and throughout Central and South America. The work of Gorgas, both in Cuba and on the Canal Zone, imitated ably and successfully by others, led to the rapid contraction of the epidemic area of this disease. Today yellow fever is known to exist only in Mexico, in a small area in Brazil, and to some extent on the west coast of Africa. That this disease can be entirely eradicated in a given locality has been demonstrated beyond a doubt. There has been no trace of this disease in Havana since the work of Gorgas and his companions. Rio de Janeiro, likewise, has remained free from every trace of it for years. There has been no case of yellow fever in Guayaquil since 1919, and, as we have stated, the possibility of banishing this disease wholly from the face of the earth seems justified. Mexico is one of the countries in which it still lingers, though only in one or two localities where a case now and then is recognized. Through the courtesy of Dr. Vasconcelos, of the Mexican Health Department, we have been kept informed concerning yellow fever in our sister republic. During the month of September, 1922, there were reported in Mexico ten cases, with seven deaths. During the month of October, nine cases, with seven deaths. This is certainly gratifying when we recall that in 1920 there were in Mexico 505 cases of recognized yellow fever, with 249 deaths, and in 1921 there were 115 cases, with three deaths.

Epidemiologists, at least some of them, say that while infectious diseases have been stamped out in certain localities, even permanently, there is no evidence that any such disease, up to the present time, has been wholly and completely banished from the earth. We know of no fact which contradicts this statement but we hope that within a few years yellow fever may be swept from the earth. —*V. C. V.*

The Preparation of Staining Solutions

IN the text books dealing with microscopy and with bacteriology, as well as in the Journal literature dealing with these subjects, there are two general types of stain formulae. In one type of formula so much dry stain in weight per given volume of solution is called for, in the other type so many cubic centimeters of a saturated solution of the stain. It will be readily seen that the same staining formula could be prepared by both methods provided the solubility of the dry stain and its actual dye content were known.

If all batches of dry stain contained the same amount of actual dye either sort of formula would be perfectly satisfactory.

Unfortunately, however, different lots of any stain vary greatly in the amount of inert material they contain. This is true even in regard to different batches of Grübler stains and it is especially true now when there are so many different brands of stains on the market. An investigation of certain methylene blues, for example, showed a Grübler sample examined to contain 57 per cent of actual dye, a Merck sample 55 per cent, while five different American samples varied from 69 per cent to 88 per cent. It seems in general desirable that the color strength should be as high as reasonably possible, and it must be particularly pointed out that the sample containing 88 per cent dye proved one of the two very best in a long series of methylene blues examined. It is obvious, however, that a staining formula calling for so many grams of Grübler methylene blue will be more concentrated if made up with one of these American stains. Considering how these stains vary in actual dye strength, much more nearly constant formulae can be obtained provided they are prepared on the basis of a definite volume of a saturated solution. Although there are undoubtedly some differences in the amount of actual dye that goes into solution in the case of different brands of stains, nevertheless, the results are much more constant in this case than when the formulae are prepared on the basis of weight of dry dye. For this reason, biologists publishing stain formulae are urged to cooperate with the committee in putting the formulae on the basis of so many cubic centimeters of saturated solution. The saturated solution may be either aqueous or alcoholic, according to the needs of the individual case. Objections are sometimes made to this type of formulae in that it is difficult to prepare without waste when the solubility of a stain is not known. This is not a valid objection, however, because a saturated solution of any stain can always be kept on hand with an excess of undissolved material at the bottom of the bottle, adding more water or alcohol from time to time as more stock solution is needed in preparing staining solutions. This type of formula, therefore, has so many advantages without serious disadvantages that its use is recommended in all possible cases.—*H. J. Conn, Chairman, Commission on the Standardization of Biological Stains.*